A Bacteriological Study of Non-tuberculous Respiratory Infections

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Margaret Alexandra Calder
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INTRODUCTION

The end of the 19th century was a period of rapid advance in the science of bacteriology. During this time a series of discoveries were made which opened a new era in the study of diseases of the respiratory system. An early important work on the bacterial aetiology of pneumonia was that of Friedländer who in 1882 observed capsulated cocci in the lung which he showed to possess pathogenic properties. Fraenkel in 1886 described capsulated diplococci in pneumonia which differed from those of Friedländer. These findings caused confusion and controversy which was cleared by the work of Weichselbaum (1886) who demonstrated there were two organisms concerned, Klebsiella pneumonia which corresponded to the organism described by Friedländer, and the pneumococcus. Although the names of Fraenkel and Weichselbaum are now linked with the pneumococcus, the first observation of the organism was probably by Klebs who in 1875 saw cocci in the lungs of patients dying from pneumonia. In 1861 Pasteur and Sternberg independantly isolated pneumococci from rabbits by the inoculation of saliva. Pfeiffer in 1892 isolated a bacillus which became known as Haemophilus influenzae in view of its association with influenza in the epidemic of 1889-92. In 1881

Ogston described the arrangement of micrococci in clusters and chains in suppurative lesions.

Thus by the end of the 19th century the organisms which are associated with non tuberculous infections of the respiratory tract had been identified but about 50 years passed before a chemotherapeutic agent was used in the treatment of these infections in man.

Pneumococcal infections in mice were successfully treated with ethylhdrocuprein by Morgenroth and Levy in 1911 but this drug was found to be too toxic for human use.

The introduction by Domagk in 1935 of the sulphonamide compounds was the first advance in the treatment of human bacterial infections with chemotherapeutic agents.

In 1928 Fleming observed the inhibitory action of a colony of penicillium on a culture of Staphylococcus aureus. This observation was the first stage in a new field of chemotherapy. The successful application of penicillin in human medicine was followed by a search for similar products and the large number of antibiotics now available, is a measure of the success achieved in this field.

Following the use of effective chemotherapy, there was a marked change in the prognosis of

respiratory infections, which is seen in the fall in the death rate from pneumococcal pneumonia. In the pre antibiotic era 13.2% of non bacteraemic and 73% of bacteraemic cases had a fatal outcome (Heffron 1939). The present death rate in pneumococcal pneumonia is now considered to be about 5% (Stuart Harris 1959).

Although much progress has been made in the treatment of non tuberculous infections of the respiratory tract, these conditions are still responsible for a great deal of sickness in the present day. Logan (1953a) showed that 24% of consultations in general practice were concerned with respiratory conditions of a non tuberculous nature. A study of the Registrar General's Review for 1959 showed that in that year, approximately 52,000 persons died from chronic bronchitis and pneumonia.

It was therefore apparent that non tuberculous infections of the respiratory tract were subjects worthy of study. The present work is devoted to an investigation of the bacteriology of these conditions with particular reference to chronic bronchitis and pneumonia.

As there have been few studies in recent years of unselected cases of pneumonia, there is

little information on the character of the disease in the present day. It was considered of interest to investigate such cases with the object of determining the nature of infections now responsible for pneumonia, and in addition to study the susceptibility of the bacteria to the antibiotics commonly used in the treatment of pneumonia.

In this country chronic bronchitis is responsible for high morbidity and mortality rates. Infection is now regarded as a factor of considerable importance in the aetiology of the disease. Repeated infective episodes produce irreversible damage to the lung tissue. In certain long established cases of chronic bronchitis, it was observed that prophylactic chemotherapy was effective in the prevention of acute excerbations of infection. It was therefore considered that useful information could be obtained from a bacteriological study of cases of chronic bronchitis where an advanced degree of respiratory disability was not present, on the degree of bacterial infection of such cases, and also if prophylactic chemotherapy was of value in curtailing the further development of the disease.

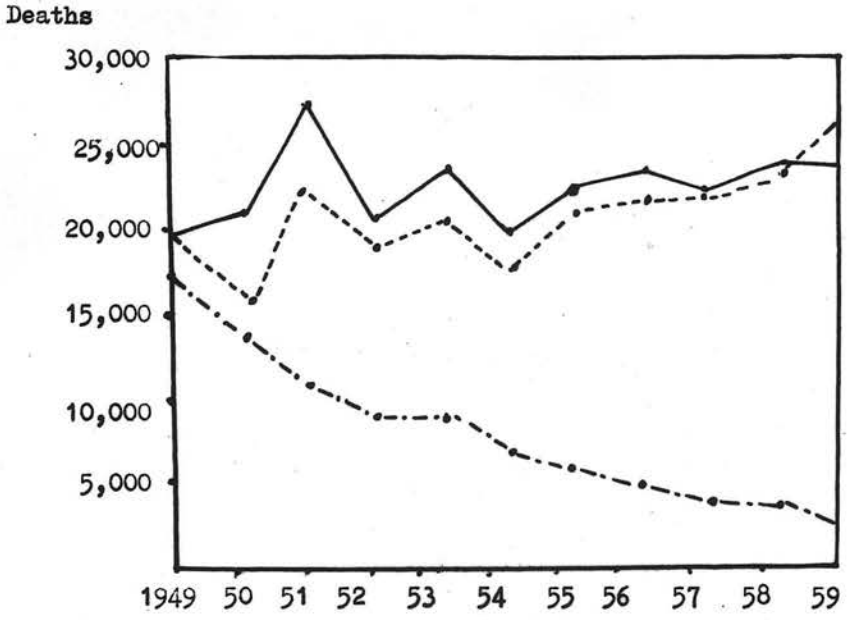
Before embarking on these investigations it was considered advisable to study certain

bacteriological methods in order to find the optimum conditions for the isolation of bacteria. These were concerned with biochemical reactions, the treatment of sputum before examination, and selective culture media. The object of this study was to develope a technique which would be applicable to the work of a busy hospital laboratory. It was considered that methods of treatment of sputum and the type of cultural media employed, would require to be relatively simple in order to cope with the large number of specimens which were expected.

CHRONIC BRONCHITIS

The serious nature of chronic bronchitis is apparent from the high mortality associated with it. In figures 1 and 2 the number of deaths which were due to chronic bronchitis in England and Wales, and Scotland over ten years have been recorded. It can be seen that chronic bronchitis was responsible for a large number of deaths each year and moreover there has been no lowering of the death rate in recent years; the deaths caused by chronic bronchitis in England and Wales in 1949 and 1959 totalled 20,166 and 24,138 respectively. This is in contrast to the steady decrease in the number of deaths due to respiratory tuberculosis which has also been

Figure 1

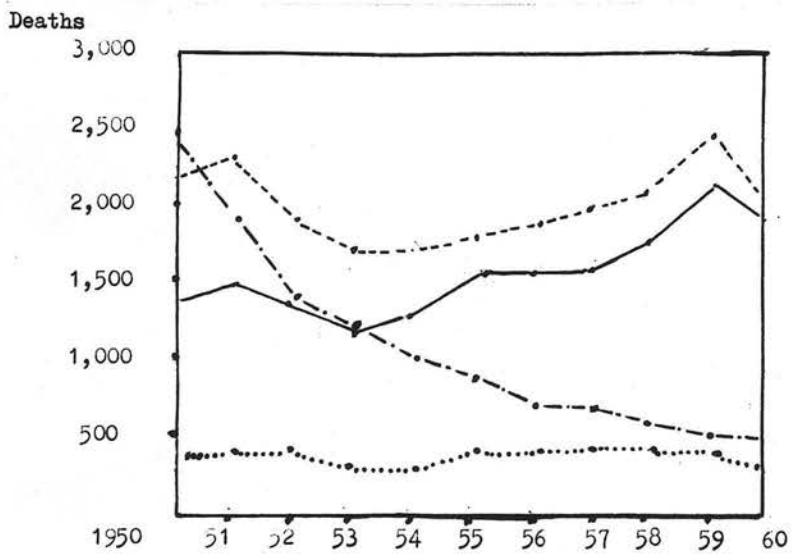


Deaths in England and Wales from —— Chronic bronchitis,

- - - - - - - Pneumonia —·— Respiratory tuberculosis

in the years 1949 - 1959 (figures to the nearest thousand).

Figure 2



Deaths in Scotland from — Chronic bronchitis

- - - - - Pneumonia ..... Lobar pneumonia

—.— Respiratory tuberculosis

in the years 1950-1960 (figures to the nearest hundred)

Lobar pneumonia is a subdivision of pneumonia. The number of deaths is therefore part of the total number of deaths due to pneumonia.

recorded on figures 1 and 2.

It is not the death rate alone of this disease which is so grave but also the illness and loss of man power resulting from it. In 1953-54 bronchitis was certified as the cause of incapacity for claimants for more than 25½ million week days. (Brit. med.J. 1956). Oswald (1958) in a study of the insured population for 1951 showed that a total of 26.61 million working days were lost due to bronchitis and this did not include mild or short lasting conditions which did not require a certificate.

Thomson (1957) found that in 1953-54 bronchitis caused 9.1 days of absence in every 100 working days.

Chronic bronchitis is so often called the English disease. Although the number of deaths caused by chronic bronchitis in England and Wales is the highest in the world, the figures for the rest of the United Kingdom give no cause for complacency. L'Eltore (1951) in a review of the percentage of deaths attributed to bronchitis in 21 European contries, found that England and Wales had the highest figure of 6.97%. The next highest figure was that of Scotland with 3.9% followed by Northern Ireland with 3.67%.

This country compares unfavourably with

Scandinavia. Stuart Harris (1957 and Ogilvie and Newell (1957) have remarked on the greater incidence of bronchitis found in England and Wales. In the period 1951-1955, Christensen and Wood (1958) found the mortality in England and Wales due to bronchitis was fifteen times that of Denmark.

Although there may be differing criteria of classification in various countries, it is generally recognised that this alone does not account for the great excess of mortality due to bronchitis in this country over that found elsewhere.

The pathogenesis of chronic bronchitis involves several factors. These include infection, polluted air, cigarette smoking, poverty, allergy, heredity, and the degenerative changes of advancing years. No one factor is primarily responsible but rather a combination of adverse circumstances.

Infection in chronic bronchitis is of a secondary nature. Conditions of reduced resistance in the respiratory tract produced and aggravated by repeated exposure to irritants such as cigarette smoke and polluted air, predispose to the invasion of the bronchial mucosa by pathogenic bacteria. Infection causes lung tissue to be damaged and replaced by fibrous tissue with

a consequent loss of pulmonary function. (Reid 1954).

It is considered that the organisms most frequently associated with the disease are Haemophilus influenzae and the pneumococcus.

Early studies of bronchitis did not show such a predominance of these organisms but demonstrated the presence of a wide range of bacteria in the sputum. This was obserbed by Marshall (1931) and Southwell (1946). In the opinion of the last writer all organisms found in the upper respiratory tract could be found in the bronchi in chronic bronchitics. Howell (1951) demonstrated a large variety of organisms in the sputum of chronic bronchitics, and in addition showed that the bacterial flora differed at subsequent examinations.

May (1952, 1953a) demonstrated that this variation was in fact due to an irregular distribution of pathogenic organisms in the sputum. He advocated for the successful isolation of Haemophilus influenzae and the pneumococcus, that the specimen should be homogenised. A technique emplying pancreatin as the homogenising agent was devised by Rawlins (1953, 1955) and used in subsequent investigations (May 1953b, May 1954, May and Oswald 1956). In 1954 May demonstrated Haemophilus influenzae in the sputum of 80% to 90% of cases. There was a variation in the isolation rate of pneumococci which fell from 71%

to 20% during the course of the study. The high isolation rate of Haemophilus influenzae was in accordance with the findings of Mulder (1952, 1956). Although Mulder did not homogenise the sputum he used a selective medium for the isolation of Haemophilus influenzae. In the majority of investigations, Haemophilus influenzae has been isolated more frequently than the pneumococcus (Elmes and others, 1953; Helm and colleagues, 1954; Edwards and associates, 1957; Buchanan and co workers, 1958; Brumfitt and Willoughby, 1958; Murdoch and others, 1959). Examination of bronchoscopy specimens from patients with bronchiectasis by Allison and colleagues (1943) showed the predominance of Haemophilus influenzae. In 1953 Stuart Harris and colleagues demonstrated a 50% isolation rate of pneumococci compared with 15% for Haemophilus influenzae.

More than one factor may be concerned with the variation in results. There is a difference in the number of cases studied in each investigation. The studies of May (1953b), Elmes and others (1953), Helm and colleagues (1954), and Buchanan and associates (1958) were concerned with small numbers of patients - 22 and less and it is difficult to relate the significance of findings in these investigations to those where large numbers of patients were involved. Such

studies were conducted by Edwards and others (1957) and Stuart Harris and associates (1953) who investigated 320 and 172 patients respectively. A possible reason for the lower incidence of pneumococci in most investigations, is the omission of a specialised technique for the isolation of this organism. It is of note that Stuart Harris and colleagues (1953) who obtained a high isolation rate of pneumococci but a low isolation rate of Haemophilus influenzae used mouse inoculation in a number of cases but did not employ a selective medium for Haemophilus influenzae. In contrast, in many of the other studies, a selective medium was used for the isolation of Haemophilus influenzae (Allison and others, 1943; Edwards and associates, 1957; Buchanan and colleagues, 1958; Murdoch and others, 1959). In addition to homogenisation, May (1954) mentioned the use of special media for subculture but details are not given. From a comparison of the bacteriological findings from bronchial swabs, sputum and throat swabs, Brumfitt and colleagues (1957) concluded that as pneumococci were frequently found on culture of throat swabs and sputum, but were absent from bronchial swabs, many of the strains of pneumococci isolated from the sputum in chronic bronchitis were contaminants from the throat. May (1958) commented that this form of bronchial swabbing would be effective in the

isolation of organisms causing inflammation at this level of the bronchial tree or deposited there by the passage of infected bronchiolar secretions, but as the site of infection in chronic bronchitis is commonly the bronchioli many organisms might not be isolated by this means. The general impression gained is that a good deal of effort has been made to isolate Haemophilus influenzae but little regard has been paid to the particular cultural requirements of the pneumococcus. Before a complete assessment of the frequency of isolation of the pneumococcus in chronic bronchitis can be made, it is essential that all methods which will enhance the growth and increase the possibility of isolation of the organism are employed. These include culture under the influence of carbon dioxide and mouse inoculation.

Further proof of the significance of Haemophilus influenzae and pneumococci in chronic bronchitis has been the removal of these organisms from the sputum after treatment with antibiotics with resulting improvement in the condition of the patient.

Chemotherapy has been employed as a preventive measure and during acute exacerbations of the disease. As Haemophilus influenzae

and the pneumococcus are both sensitive to the action of tetracycline this drug has been successfully used in long term chemotherapy for the prevention of acute excerbations of infection. (May and Oswald, 1956; Edwards and colleagues, 1957; Buchanan and associates, 1958; Murdoch and others, 1959). The investigation by Murdoch and others, (1959) which was a double blind trial was carried out in two successive winters. It was observed during the second winter when there was a high incidence of fog, that these climatic conditions were associated with a high isolation rate of Haemophilus influenzae with purulent sputum, and the largest number of acute excerbations of infections in both groups of patients occurred during this period. Elmes and colleagues (1955) considered that patients infected with Haemophilus influenzae were more susceptible to the action of fog.

Successful treatment of acute excerbations with tetracycline was reported by some writers (Mulder and associates, 1952; Helm and colleagues, 1954; Douglas and others, 1957). Penicillin alone in high doses and together with streptomycin was effective in a high proportion of cases of Haemophilus influenzae infection studied by Mulder and associates (1952). May (1956)

found penicillin effective against the pneumococcus but Elmes and associates (1953) considered that this treatment resulted in an overgrowth of Haemophilus influenzae. It was observed by Mulder and colleagues (1952), May (1953b), and Elmes and associates (1953), that infections with Haemophilus influenzae responded initially to Chloramphenicol but relapses were frequent.

It is apparent that not all cases benefit from antibiotic treatment. As Haemophilus influenzae appears to be frequently resistant to treatment, further studies should be directed towards the eradication of this organism from the sputum. However the choice and dosage of treatment may be limited by the presence of side effects as with tetracycline and oxytetracycline, and the risk of blood dyscrasias with chloramphenicol. A new antibiotic, ampicillin has recently been produced. As it is considered to be effective against Haemophilus influenzae and the pneumococcus, future studies on the treatment of chronic bronchitis with this drug are awaited with interest. The importance of the control of infection cannot be emphasised enough, as each exacerbation adds further irreversible damage to the lung tissue.

Further evidence of the infective role of Haemophilus influenzae in chronic bronchitis has

been the demonstration by some authors of antibodies to this organism in the serum of patients suffering from the disease (Murdoch and colleagues, 1959; Brown and Wilson, 1959; Glynn, 1959). In the study of Brown and Wilson (1959) no details were given of results obtained or the techniques employed. Murdoch and colleagues (1959) gave no particulars of the method used but stressed the importance of raised titres in patients with chronic bronchitis. Much higher titres were obtained by Glynn (1959) who detected antibodies by titration against tanned red cells. He found no relation between the antibody titre and the presence of infection. It is considered that further detailed study is necessary, and a series of examinations in both the quiescent and the acute infective stage of the disease could yield information of the degree of infection and the nature of the patient's response to it.

The question arises of the origin of the infecting bacteria and the factors concerned with the initiation of the inflammatory process.

From a consideration of the types of pneumococci isolated in chronic bronchitis, it would appear that the infection is endogenous. The pneumococcal types in chronic bronchitis as in

bronchopneumonia, are similar in distribution to those isolated from the naso pharynx. This finding was observed by Stuart Harris and colleagues (1953). Heffron (1939) in a review of the pneumococcal types found in carriers, showed the distribution to be type 1, 0.4% type 2, 0.8% type 3, 10.5% and the higher types 34.9%. Types of pneumococci commonly found in healthy people are 6, 19, and 23 (Cruickshank and others 1960). Masters and colleagues (1958) found that type 14 was commonly found in carriers.

Haemophilus influenzae is also found in the naso pharynx of healthy people. A carrier rate of from 40 to 80% was found by Rosher (1939). In the study by Masters and associates (1958), an isolation rate of from 50 to 70% was found in the perinasal swabs and throat swabs of the families who were investigated.

It is apparent there is a resevoir of potentially pathogenic organisms, but it is less clear what actuates and facilitates invasion of the respiratory tract.

The relationship between infection and mucus is of importance. Oswald (1958) considered it and essential factor in the aetiology of bronchitis. Mucus is derived from the goblet cells and mucous glands. In bronchitis the goblet cells in the smaller bronchi and

bronchioles become more numerous and in consequence there is a reduction of the ciliated cells. This in turn leads to a stagnation of the viscid secretion which is not removed by the ciliary streams. Suitable cultural conditions are thus produced for the organisms inhaled from the upper respiratory tract. The secondary nature of infection in chronic bronchitis is thus a failure of defence of the lower respiratory tract against invasion by these organisms.

Hypersecretion of mucus is stimulated by atmospheric irritants and infections.

Two common irritants of the respiratory tract are air pollution and cigarette smoking.

That air pollution and fog have a deleterious effect on the bronchial tree has been appreciated for a long time. Mortality in the urban areas is greatly in excess of that in the rural districts. The highest figures are in Yorkshire, Lancashire and South Wales followed by Tyneside, the Midlands and London. (Oswald 1958). In Scotland where the four cities of Glasgow, Edinburgh, Aberdeen and Dundee account for approximately one third of the population of the country, the number of deaths in 1959 from chronic bronchitis in the four cities was 49% of the total of 1987 deaths from chronic bronchitis in Scotland in that year, which is an indication of the association of industrial

surroundings with the disease.

An additional hazard of industrial areas is the presence of fog. During the London fog of 1952, the number of deaths from bronchitis was greatly increased. There was an eight fold rise recorded in the two weeks following the clearing of the fog, after which time the number of deaths fell although the level remained high for several weeks (Logan 1953b). Martin and Bradley(1960) studied the correlation of mortality, fog and atmospheric pollution in the winter of 1958-59 in London. These writers found that the proportion of deaths certified as caused by bronchitis was small in comparison with the increases in total mortality, and considered that in future, investigations in this field should take into consideration the association between respiratory and cardiac diseases. Figures were quoted that 25% to 41% of cases with congestive cardiac failure were suffering from cor pulmonale due to chronic pulmonary disease, and that more cases of cor pulmonale were associated with an industrial environment than with other surroundings. As irritation of the bronchial tree by the inhalation of polluted air may be an initiating factor in the pathogenesis of chronic bronchitis, studies on the nature of the irritant elements may lead to a better understanding of the problem.

Tobacco smoking has been shown to have an

association with chronic bronchitis. Doll and Hill (1956) found a ten fold increase in mortality in patients with chronic bronchitis who were heavy smokers. An investigation into the smoking habits of clerical Civil Service employees showed a higher prevalence of chronic bronchitis among smokers, and this was present in both males and females to an equal extent (Oswald and Medvei 1955). Ogilvie and Newell (1957) compared the smoking habits of an approximately equal number of bronchitic patients and non bronchitic controls and showed that in both sexes the bronchitics were the heavier smokers. In a study of respiratory symptoms in men in an industrial town, by Higgins and colleagues (1956), it was demonstrated that symptoms of chronic bronchitis were more frequent among smokers than non smokers. A later study by Higgins (1959) in a larger group of men, showed the same correlation between chronic bronchitis and smoking. He also demonstrated that tobacco smoking interfered with pulmonary ventilation. In the assessment of the association between tobacco smoking and bronchitis, it is necessary to take into account other related factors. These include pollution of the atmosphere, the age of the patient, the presence of infection, and climatic conditions. Certain of these were considered by Christensen and Wood

(1958) when reviewing the difference in mortality rates for bronchitis in England and Wales, and Denmark. The excess amount of cigarette smoking in England and Wales was regarded of possible significance.

Bronchitis would appear to be a condition affecting the male to a greater extent than the female population. In 1959 there were 16,540 deaths in males and 6,730 deaths in females. It is also a disease affecting the older age groups. In 1959 89% of deaths occurred in patients over the age of 60. Between the age of 50 and 60 one man in twelve is certified as having died from chronic bronchitis (Oswald 1958). It might appear that the smoking habits of the older age group would be of importance, and in view of the now increased incidence of cigarette smoking in females, future morbidity and mortality rates may reveal further information of the significance of this habit.

Until recently there was scanty evidence of the role played by virus infections in the aetiology of chronic bronchitis. Mulder (1952) and Hers and Mulder (1953) considered that infection with Haemophilus influenzae could be primary,or in the nature of a secondary invasion following necrosis of the bronchial epithelium by

a virus. Murdoch and others (1959) found rises in titres to Influenza A in one third of the patients studied. A rise in titre to influenza A has also been observed in a study on chronic bronchitis at present being conducted at the City Hospital, Edinburgh. Of interest has been the recent observation (unpublished) by Somerville of Glasgow of diagnostic titres and rises in titres to the Respiratory Syncytial virus in adults over the age of 50 years. Further work in this field is necessary. The common cold is a frequent precursor of an acute exacerbation, and it may be that future studies on this subject may reveal information of value.

PNEUMONIA

In recent years less notice has been paid to acute infections of the respiratory tract, which may be due in part to the marked change in the prognosis of these conditions since the introduction of sulphonamide therapy. These conditions are responsible for a great deal of sickness in this country. In the opinion of the College of General Practitioners (1956), one million people in Great Britain each year, suffer from an acute chest infection. Fry (1960) studied the incidence of such infections in general practice

over a five year period,and observed that 462 out of 4,673 persons in the survey suffered episodes on pneumonia or acute bronchitis during this period, and 15% of these illnesses were diagnosed as pneumonia.

Of the acute infections of the respiratory tract,pneumonia is the most serious and a study of the death rate from this disease shows that it is responsible for a large number of deaths. This is evident from the figures presented on figures 1 and 2 where the number of deaths due to pneumonia in England and Wales, and Scotland over ten years have been recorded. A disturbing feature is that the high levels have been maintained throughout the years.

Bronchopneumonia was responsible for the highest mortality; of 26,590 deaths certified as due to pneumonia in England and Wales in 1959, 21,842 were cases of bronchopneumonia. A study of the age groups showed that this was a disease of danger to the very young and the elderly. There were 1,279 deaths in children aged from one month to one year and 18,219 deaths occurred in patients over the age of 60 years. It was observed in these older patients that the number of deaths increased progressively with age. For example there were 3,002 deaths between the ages

of 60 and 70 years, 6,965 deaths between the ages of 70 and 80 years and 8,252 deaths over the age of 80 years.

Lobar pneumonia caused 3,190 deaths in England and Wales in 1959 and again the older age groups were affected, 79% of deaths occurring over the age of 60. It can be seen from figure 2 that in Scotland the death rate from lobar pneumonia has remained comparatively constant over ten years.

Consideration of the sex distribution in mortality indicated that both males and females were affected to the same extent.

The majority of patients recover from an acute infection of the respiratory tract and the question arises of what happens to these people. Do they recover completely or are there residual after effects? This problem was studied by Fry (1960) who reviewed 424 patients who had suffered from pneumonia or acute bronchitis 5 to 10 years previously. It was found that during this time 44% of the patients had had a recurrence of an acute chest infection. The group of patients most affected were males under 10 years and over 50 years. The amount of disability was estimated by assessment of function and the degree of residual symptoms. It was estimated that 43% of patients were disabled, and of these 9% were

compete invalids. The greatest amount of disability was found in the patients who had suffered from acute bronchitis.

The nature of the infecting agent in these cases and the type and amount of treatment given would have been of interest as a residual infection is a possible reason for a proportion of these cases becoming chronic pulmonary invalids.

It is apparent that pneumonia remains a problem, and it is therefore pertinant to enquire further into the character of the disease now present, and if there has been a change in the type of infecting agent.

Pneumonia is an inflammation of the lung with the production of an exudate in the alveoli. In the past it has been customary to divide pneumonias on an anatomical basis into lobar pneumonia, and lobular or bronchopneumonia. The nature of the pneumonic lesion is associated with the character of the infecting bacterial agent and the inflammatory reaction produced by the organism. In lobar pneumonia there is an inflammatory consolidation in response to an acute infection by a specific organism of a virulent nature frequently a pneumococcus. The lower pneumococcal types are most commonly

responsible for infection in lobar pneumonia. Where the consolidation is less extensive and part of the lobe is affected, the term segmental pneumonia has been applied. In bronchopneumonia also called lobular pneumonia, there are scattered areas of inflammation in the lung which may become confluent. This condition is generally in the nature of a secondary infection by organisms which may be of relatively low pathogenicity, in patients where lowered conditions of resistance exist. The association of this condition with reduced resistance is illustrated by the high incidence found in young children and elderly people. Pneumococcal types found in this disease are commonly those found in the naso pharynx, indicating that the infection is endogenous in nature. Although the infecting organism in the majority of cases of bronchopneumonia is the pneumococcus (Cruickshank 1933), it may be found in combination with other bacteria such as streptococci, Staphylococci, Klebsiella pneumoniae, and Haemophilus influenzae, and difficulty may arise in deciding which organism is primarily responsible for the pneumonia.

It is evident from the present literature that the classification of pneumonia has undergone a change in recent years. Many authors no longer adhere to the terms lobar and bronchopneumonia but

prefer to regard the illness along aetiological lines.

PNEUMOCOCCAL PNEUMONIA

When the bacterial aetiology of pneumonia is considered, it is seen from recent studies that the pneumococcus was the organism isolated most frequently. There was however some variation in the results obtained by these investigators.

In 1951 Crofton and co workers isolated pneumococci from 29 out of 129 cases and Stuart Harris (1953) isolated pneumococci from 68% to 80% of cases in a series of investigations. Grist and others (1952) isolated pneumococci from 84 of 129 patients, 79% of the patients in the investigation by Humphrey and colleagues (1948) were considered to have a pneumococcal pneumonia but a bacteriological diagnosis was not made on 29% of these cases.

Other recorded isolation rates of pneumococci are 60% (Joules and Weller 1947), 67% (Eadie and colleagues 1951), and 73% (Committee of the Antibiotic Clinical Trials of the Medical Research Council 1951).

In view of the extreme sensitivity of the pneumococcus to a wide range of antibiotics, the continued mortality from pneumococcal infections

have led certain investigators to enquire further into possible reasons for such deaths. Israel and colleagues (1948) surveyed three series of cases from 1936 to 1946. There was an association between increasing age and increased mortality which was made more marked by a rise in the age of patients suffering from pneumonia. Van Metre (1954) and Dowling and Lepper (1951) also found the death rate rose with the age of the patient. This is not a new observation and the survey by Heffron (1939) showed that the death rate from pneumococcal pneumonia rose progressively with age. It would appear that despite the appropriate treatment certain elderly patients are still unable to withstand the effect of a pneumococcal infection, which would appear to be of significance in view of the increasing age of the population in general.

When pneumococcal infections are considered, the type of the infecting pneumococcus is important, as the presence of the more virulent types is an indication of the serious nature of the disease. In a wide review of the literature, Heffron (1939) demonstrated that the most frequent infecting types in lobar pneumonia were types 1,2 and 3 in that order, and of these types, 1 and 2 accounted for 52.2% of infections. Some more

recent studies have shown that type 1 and 2 remain the commonest infecting types. A predominance of type 1 infections has been found in some studies (Humphrey and others,1948; Israel and associates, 1948; Austrian and Winston, 1956; Thomson and colleagues, 1951; Van Metre, 1954). In the investigations of Anderson and Ferguson (1945), Anderson and Landsman (1947), Eadie and associates (1951) and Grist and colleagues (1952) the commonest pneumococcal type isolated was type 2. It is of note that these investigations were undertaken in Glasgow. Earlier studies in this city reviewed by Heffron (1939) also showed a high isolation rate of this type indicating a regional distribution. A predominance of type 2 pneumococci was found by Hodges and MacLeod (1946) who studied epidemic pneumonia in an Army Air Force camp. Although this investigation illustrates the tendency of this type to infect young adults, the frequency of isolation was increased by the epidemic nature of the disease.

The work of Heffron (1939) demonstrated the high mortality rate in pneumococcal infections caused by type 3 pneumococci and the tendency of this type to infect elderly people. On consideration of later studies where the infecting type has been considered in relation to these

factors, it would appear that type 3 infections remain the most serious. Thompson and others (1951) were of this opinion and observed the fatal nature of such infections in elderly people. It has been found that the highest mortality in pneumococcal pneumonia was in cases infected with type 3 pneumococci (Van Metre, 1954; Dowling and colleagues, 1947; Humphrey and associates, 1948). A slight predominance of type 3 strains in fatal cases was observed by Dowling and colleagues (1950). Of interest in this study was the high incidence of type 8 infections, as this type is closely related immunologically to type 3 and like it, possesses a large capsule.

In the opinion of Thompson and associates (1951), the record of virulence enjoyed by the type 3 pneumococcus was due to its tendency to infect elderly people as it was an infrequent invader of the blood. Cruickshank (1933) considered that although the type 3 pneumococcus was not invasive in character, the toxaemia produced was severe, and this was present in both young and old patients alike. In this connection he considered of importance the inhibiting action of the polysaccharide on phagocytosis and possibly antibody production.

The presence of pneumococci in the blood has always been considered a bad prognostic sign.

Heffron (1939) considered that the presence of antibody was of importance in the prevention of the appearance of bacteraemia. In a review of the mortality in cases of bacteraemia, he found this to be 61.8%, and where the infecting type was type 3 the death rate ranged from 86.1% to 100%.

In recent studies although the mortality has fallen greatly, the association between mortality and bacteraemia particularly if this is due to type 3 pneumococci, is still present.

The low invasive nature of the type 3 pneumococcus has already been mentioned. Heffron demonstrated that the most common types found on culture of the blood were types 1 and 2 and of these type 2 was most frequently isolated.

From a consideration of more recent studies it would appear that these types are still most frequently isolated from the blood. Type 1 was predominant in some investigations (Dowling and others, 1947; Gocke and associates, 1949; Dowling and colleagues, 1950). Israel and co workers, (1948) surveyed three series of cases from 1936 to 1946 and found that while type 1 was the commonest type in the earlier years, it was replaced by type 2 in 1946. In the study by Anderson and others (1947) in Glasgow the predominance of type 2 was marked. A later

study in the same city by Eadie and colleagues (1951) demonstrated to a lesser extent a higher isolation of type 2 pneumococci as compared with other types.

The percentage of cases of lobar pneumonia showed by Heffron (1939) to have a positive blood culture was 28.8%. On consideration of more recent investigations, it is seen that bacteraemia is still a complication of pneumococcal pneumonia. Some recorded rates of this condition are 24.7% (Austrian and Winston 1956), 12.9% (Thomson and others 1951), 17% (Ahern and Kirby 1953), and 5% (Eadie and colleagues 1951).

It can be seen there is a variation in the percentage of cases with a positive blood culture in the different investigations. This could be influenced by the type of case selected for study but there is insufficient information to decide if this is of significance. However, this survey does indicate that in the antibiotic era, pneumococci may be cultured from the blood not infrequently in pneumococcal pneumonia. This is of importance as there is an increased mortality associated with this condition which is evident from consideration of the work of investigators in this field.

In bacteraemic cases a mortality rate of 9% was found by Van Metre (1954) The figure given

by Dowling and others (1951) was 11% and by Dowling and Lepper (1951) 13%. There were seven deaths in the study by Anderson and Landsman (1947) and in six of these pneumococci were cultured from the blood. A positive blood culture was obtained from eight of the twenty two fatal cases in the investigation by Anderson and Ferguson (1945). Austrian (1959) and Israel and colleagues (1948) commented on the association between bacteraemia and mortality.

When the transmission of pneumococcal infections is considered, it is seen this is a complex subject and there are many associated factors. The method of infection may be endogenous or exogenous, which is associated with the carrier rate of different types of pneumococci in the population. The carrier rate of the first three types is given by Heffron (1939) as type 1 0.4%, type 2 0.8% and type 3 10.5%. In a study of the naso pharyngeal flora, Straker and colleagues (1939) found a carrier rate of pneumococci of 20-40% in the population and type 3 was isolated frequently in this study. Types 6, 19 and 23 are commonly found in the upper respiratory tract of carriers (Cruickshank and colleagues 1960). Masters and associates (1958) found type 14 was also commonly found in the

carrier state. The low carrier rate of types 1 and 2 in contrast to the high incidence of pneumonia caused by these types, has led to the opinion that infection in these cases is exogenous. Heffron (1939) considered that where pneumococci are isolated as frequently from carriers as from cases of pneumonia, the infection can be considered to be endogenous in nature. This is a general ruling and without a study of contacts it is not possible to determine the method of infection.

The family contacts of pneumonia patients were reviewed by Heffron (1939) where it was seen that close family contacts quickly became carriers of the infecting type of pneumococcus. In a study of the spread of respiratory disease among families, Brimblecombe and colleagues (1958) found that the transfer rate of pneumococci isolated from the nose and throat rose significantly with the degree of overcrowding. Where new types of pneumococci were introduced into the family, it was found that school children and fathers, the members of the group with most outside contacts, were the most frequent offenders. Hodges and MacLeod (1946) studied epidemic pneumococcal pneumonia in an Air Force camp. A study of the carrier rate showed that new

arrivals at the camp rapidly acquired the infecting types during the first six weeks of their stay in camp. There was a good correlation between the carrier rate of certain of the infective types of pneumococci and the incidence of pneumonia caused by these types.

It is apparent that overcrowding is conducive to the spread of pneumococci. The presence of the carrier state alone is not responsible for the onset of pneumonia. A lowering of the resistance of the host is required and several factors may influence this. It is considered in many cases a preceding upper respiratory infection has facilitated invasion of the lower respiratory tract by pneumococci. There is a seasonal influence which may be due to two factors, the higher pneumococcal carrier rate in the winter months and the increased incidence of upper respiratory infections during this period. Other precipitating factors include malnutrition, exposure to wet and cold and alcoholism.

All these conditions have been shown in the past to predispose to infection with the pneumococcus. There is less information on the extent of the influence of such factors on infection in the present day. From the investigation of Fry (1960), it would appear that lobar pneumonia remains more frequent among the

lower social groups.

STAPHYLOCOCCAL PNEUMONIA

Apart from its appearance with influenza, Staphylococcus aureus is not a frequent cause of pneumonia. Where the infection is not superimposed on a viral lesion there is often evidence of a pre existing pulmonary condition.

The results of surveys of pneumonia undertaken when influenza was not prevalent, demonstrate the incidence of staphylococcal pneumonia during such periods. The incidence found by Crofton and colleagues (1951) was 9.1% by Humphrey and associates (1948) and by the Committee of the Antibiotic Clinical Trials of the Medical Research Council (1951) 3%. Stuart Harris (1953) studied four series of cases, two in the non influenzal period and two when influenza was prevalent. The percentage of staphylococcal pneumonias in the first non influenzal period was 5.3% but in the influenzal period the figure rose to 29%. This result compares with the percentage of 27% of staphylococcal pneumonias found in the investigation of pneumonia during an epidemic of influenza by Robertson and others (1958).

The serious nature of staphylococcal pneumonia is reflected in the high mortality associated

with it. In the investigation by Crofton and colleagues (1951) there was a 40% mortality in cases of this disease. Of the seven patients with staphylococcal pneumonia in the series by Humphrey and others (1948) five died. In these cases there was not evidence of a previous virus infection although one of the patients studied by Crofton and colleagues (1951) died from the fulminating type of illness associated with a combined infection.

Hers and associates (1958) studied the bacteriology of 158 fatal cases of influenza. It was found that Staphylococcus aureus was the most frequent secondary invader and was isolated from 60% of cases. In an investigation of pneumonia during an epidemic of influenzae by Robertson and others (1958), Staphylococcus aureus was isolated most frequently and was responsible for the highest mortality, 47% as compared with 16% in the remainder of the cases. A higher isolation rate of Staphylococcus aureus as compared with other organisms was found by Walker and co workers (1958) in a similar study. In this study a bacteriological diagnosis was made in only 46% of cases, which was probably due to 40% of the patients having received treatment before admission. This could also have resulted in

failure to isolate more susceptible organisms particularly the pneumococcus. Stuart Harris (1953) considered that Staphylococcus aureus was less frequently involved than the pneumococcus as a secondary invader in influenza. It was observed by Walker and co workers (1958) that all cases of fulminating pneumonia were infected by the Staphylococcus aureus.

The association of a combined viral and staphylococcal infection with a severe type of pneumonia might indicate that each organism potentiates the action of the other. Stuart Harris (1953) postulated the existance of a degree of synergism but there is no proof of this.

The characteristic feature of this type of pneumonia is the destruction of the columnar epithelium of the trachea and bronchioles. In the opinion of Mulder (1952), the destruction of the epithelium by the virus enables the staphylococci to gain entry to the mucosa.

The source of a secondary staphylococcal infection may be endogenous or exogenous. It is estimated that half the population are nasal carriers of staphylococci. Such people run a risk, as the possible consequence of lowering of the resistance of the lower respiratory tract by influenza or other inflammatory conditions is an

acute staphylococcal infection. The patient in hospital has the additional hazard of infection by staphylococci from the hospital environment. This was shown to be the source of infection in 46% of cases of staphylococcal respiratory infections in one hospital studied by Maccabe (1959). Robertson and colleagues (1958) considered that one third of cases of staphylococcal pneumonia in the investigation were infected in hospital. These two studies were conducted during a period when influenza was prevalent. The marked degree of cross infection found in these investigations is in contrast to the low rate observed by Shooter and associates (1960) who studied cross infection when influenza was not prevalent. It was estimated that six of 349 patients acquired a staphylococcal sepsis from a hospital source, although many patients were regarded as having conditions which might have predisposed to a staphylococcal infection. Although this would demonstrate the importance of influenza as a predisposing factor, the problem of cross infection is a complex one. Not all staphylococci possess the same virulence. Of danger to the patient are the types possessing multiple resistance to antibiotics as these have survived from treated patients and have remained

in the hospital environment. The danger of indiscriminate treatment with a wide range of antibiotics was stressed by Walker and colleagues (1958) in view of the possible colonisation of a sterilised upper respiratory tract with these resistant strains.

HAEMOPHILUS INFLUENZAE

It is considered that pneumonia due to pure infection with Haemophilus influenzae is a rare occurrence. Of 3189 cases of lobar pneumonia reviewed by Heffron (1939), 0.2% were considered caused by infection with Haemophilus influenzae. A similar survey was carried out more recently by Crowell and Loube (1954) who found Haemophilus influenzae the infecting agent in four out of 3,600 cases of lobar pneumonia. Mulder (1952) stated that he had never isolated Haemophilus influenzae in pure culture from any cases of lobar or segmental pneumonia.

From the presence of Haemophilus influenzae in large numbers at post mortem examination of the lungs of cases of influenza in the 1889-92 epidemic, Pfeiffer considered that this organism was the cause of the disease, and so the name Haemophilus influenzae was given. Although subsequent studies have shown that the primary

infection in influenza is viral in nature, the appearance of Haemophilus influenzae in the lung tissue, would illustrate its secondary infective role.

Haemophilus influenzae may be found together other organisms in cases of pneumonia where there are scattered areas of consolidation, frequently peribronchiolar in distribution (Scadding 1952; Reimann 1938).

There are several factors which may cause uncertainty in making a diagnosis of Haemophilus influenzae pneumonia.

Bacteriologically the diagnosis may be obscured by the presence of other bacteria, particularly pneumococci. In such cases the problem of assessing the relative pathogenicity of these organisms arises, which is of importance in view of the differing susceptibility of each to penicillin. It would be inadvisable to ignore the presence of Haemophilus influenzae as treatment with penicillin alone would allow the drug resistant Haemophilus influenzae to further colonise the respiratory tract. This was demonstrated in the treatment of chronic bronchitis with penicillin by Elmes and colleagues (1953).

Rosher (1939) demonstrated that 40-80% of the population carried Haemophilus influenzae in the

naso pharynx and it is the presence of these organisms in healthy people which has led to a certain amount of doubt of the significance of strains of Haemophilus influenzae isolated from the sputum.

Associated purulence of the sputum, and the degree of growth of the organism on culture may be indications of its significance as an infecting agent. The degree of growth can only be estimated accurately by the use of a selective culture medium. For the supply of necessary growth factors, Haemophilus influenzae must be cultured on a medium containing blood. However some strains of Haemophilus influenzae do not grow on ordinary blood agar and better results are obtained by the use of heated blood agar. In the investigations of Stuart Harris and colleagues (1953) and Humphrey and associates (1948), the use of ordinary blood agar was considered to have contributed to the failure to isolate some strains of Haemophilus influenzae.

KLEBSIELLA PNEUMONIAE

Pneumonia due to Klebsiella pneumoniae is uncommon. In a review of the early literature, Heffron (1939) showed the incidence to be 0.5 to 0.6% of pneumonias although a higher incidence of 10% had been observed by some authors. Present

day studies show that this form of pneumonia is still a rare occurrence. Of 351 cases of pneumonia studied by Humphrey and colleagues (1948), two were due to Klebsiella pneumoniae. There were no cases of this form of pneumonia in the study by Stuart Harris (1953) undertaken in the non influenzal period. Three of 166 cases of pneumonia investigated by this author during a time when influenza was prevalent, were due to infection with Klebsiella pneumoniae. A 1% incidence was found in the investigation by the Committee of the Antibiotic Clinical Trials of the Medical Research Council (1951), and in the study by Joules and Weller (1947). No cases were reported from the studies by Eadie and colleagues (1951) and Crofton and associates (1951)

Although this form of pneumonia is rare it is responsible for a high mortality. In the pre antibiotic era death occurred in the majority of cases. Heffron (1939) reported mortality rates of 63.3% to 90%. Although the death rate has fallen since the introduction of antibiotics, it still remains at a high level. Jervey and Hamburger (1957) in a review of cases during the years 1948 - 1956 found the death rate to be 53%. Two of the three cases in the investigation by the Committee of the Antibiotic Trials of the Medical

Research Council (1951) ended fatally as did one of the two cases in the study by Humphrey and colleagues (1948).

The illness produced by Klebsiella pneumoniae is of a severe nature. There is necrosis and destruction of the lung tissue which may result in abscess formation. A proportion of the patients who recover from the acute form of the illness, develope a chronic infection which results in a prolonged illness associated with fibrosis and abscess formation.

Pneumonia due to Klebsiella pneumoniae is found most frequently in males in the older age groups. Such patients are generally debilitated and alcoholism is considered the most common precipitating factor (Heffron 1939; Reimann 1938). The presence of the organism in the naso pharynx of healthy people may cause difficulty in assessing the significance of isolations of Klebsiella pneumoniae from the sputum of patients with an infection of the lower respiratory tract. Weiss and associates (1956) studied the carrier rate of Klebsiellae in several groups of people. It was found that the carrier rate in the naso pharynx in patients, particularly inpatients, with respiratory disease was higher than in healthy people.

Pneumonias of a non specific bacterial aetiology

The presence of a group of pneumonias where no specific bacterial pathogen was recovered has been observed in recent years. This is demonstrated in the results of studies of unselected cases of pneumonia. In the series of cases investigated by Crofton and others (1951) 47% were not diagnosed bacteriologically. 15% of the cases studied by Humphrey and associates (1948) could not be attributed to a specific bacterial infection. In the investigation by the Committee of the Antibiotics Clinical Trials of the Medical Research Council (1951), 20% of the cases were not diagnosed bacteriologically. In the study by Batty Shaw and Fry (1955) a bacterial pathogen was isolated from nine of forty two cases of pneumonia. Grist and others (1952) commented on the percentage of cases - 27% from which no pathogenic organisms were isolated.

An obvious reason for failure to isolated the infecting agent is masking of the organism by previous antibiotic treatment. Crofton and colleagues (1951) considered that 15 cases of pneumococcal pneumonia were possibly not diagnosed bacteriologically for this reason. In the investigation by the Committee of the Antibiotics Clinical Trials of the Medical Research Council

(1951), patients who had received penicillin or sulphonamide were not accepted but treatment with other drugs is a possibility. 14.5% of the patients in the study by Humphrey and colleagues (1948) had received sulphonamide drugs before admission and of these more than half had received less than 10g. By far the largest number of specimens examined during the investigation by Batty Shaw and Fry (1955) were received before any treatment was given. The low isolation rate of pathogenic organisms is therefore surprising and may be partly explained by the fact that sputum specimens were not received from all patients. In these cases throat swabs and perinasal swabs were substituted. Cases of acute bronchitis and influenza with chest complications were also studied and it is not clear which type of patient failed to produce a sputum specimen. A specimen of sputum was received from fifty one of the eighty patients studied.

Although previous antibiotic treatment may account for a failure of diagnosis in certain cases of unknown bacterial origin, it is not the complete answer. Stuart Harris (1959) commented on the decline of the bacteriologist's ability to identify the cause of presumed bacterial pneumonia.

This decline might be associated with failure to employ all available means of identification of possible infecting bacteria.

As has already been discussed the pneumococcus remains the most common cause of bacterial pneumonia. This is an organism which by ordinary cultural techniques may remain undetected. May (1952 1953a) demonstrated that pathogenic bacteria may be irregularly distributed in the sputum and advised homogenisation of the specimen for satisfactory isolation of Haemophilus influenzae and pneumococci. Of the above investigators only Grist and colleagues (1952) appeared to have employed this technique.

The use of a selective medium may result in an increased isolation rate of pathogenic organisms. This was demonstrated by Master and others (1958) where the use of an enrichment medium resulted in a higher yield of pneumococci. Haemophilus influenzae may fail to grow on ordinary blood agar. Humphrey and colleagues (1948) and Stuart Harris and associates (1953) were of the opinion that if a selective medium had been employed, a higher isolation rate of Haemophilus influenzae would have been obtained.

It is of note that heated blood agar was used in the investigation by Holland and others

(1960) where the isolation rate of Haemophilus influenzae of 22% was higher than found in comparative studies where no selective medium was employed.

The addition of carbon dioxide to the atmosphere is beneficial to the growth of pneumococci. Humphrey and colleagues (1948) demonstrated this in a parallel series of cases. In 1941 Fleming described a strain which grew only under such cultural conditions.

Mouse inoculation is a valuable aid in the isolation of organisms particularly pneumococci. It was considered by Crofton and co workers (1951) that certain strains of pneumococci were missed as mouse inoculation was not performed throughout the investigation. Humphrey and others (1948) employed this technique in certain cases and came to the opinion that by its use the isolation rate of pneumococci could be increased by 35%. It is of interest that in the investigations by Grist and colleagues (1952) and the Committee of the Antibiotic Clinical Trials of the Medical Research Council (1951), where mouse inoculation was performed, there were comparatively high isolation rates of pneumococci.

It is apparent that the use of specialised techniques may improve the isolation rate of pathogenic bacteria. Austrian (1959) commented

on the abandonment of such methods by most laboratories.

In the study by Batty Shaw and Fry (1955) where the investigation was carried out in general practice, comment was made of the poor character of the sputum specimens received at the laboratory and the delay associated with laboratory reports which rendered many of these worthless. As the circumstances vary greatly from hospital practice, it is difficult to comment fully, but it is considered essential there is the fullest co-operation between the clinician and the laboratory worker in order that the most suitable specimen is received for examination. An earlier indication of the diagnosis could have been obtained from examination of a Gram film. Such an investigation is not time consuming and is in addition a useful guide to the bacteriologist in deciding which specialised techniques may be advisable.

Even with the employment of selective techniques, there remain pneumonias from which no specific bacterial infecting agent can be isolated. These cases have led to the introduction of the term 'aspiration pneumonia' by Scadding (1952). These occur during the course of acute and chronic catarrhal infections of the upper respiratory tract and are associated with debilitating conditions where the resistance

is lowered. Where the defence mechanism of the lower respiratory tract is impaired, aspiration of the secretions of the upper respiratory tract results in an inflammatory reaction. This is not an infrequent occurrence in elderly particularly bedridden patients who are unable to expectorate the bronchial secretions. In the investigation by Crofton and colleagues (1951) it was considered that 'aspiration pneumonia' was a probable diagnosis in about half the cases where no specific organisms was isolated. A diagnosis of 'aspiration pneumonia' was made in twenty nine of the forty two cases of pneumonia studied by Batty Shaw and Fry (1955).

In recent years increasing attention has been paid to fungal affections of the respiratory tract, which appear to have increased in incidence. The fungi which may be responsible for human infections are many; this review however is concerned with the fungal conditions most commonly encountered in Edinburgh.

ASPERGILLOSIS

In this country the most important pulmonary condition due to fungi is aspergillosis (Riddell 1956)

This infection may be primary but is usually

of a secondary nature complicating an existing lesion of the lung, Riddell (1961) described two forms of the disease. The first he termed saprophytic, where the fungus grows in damaged lung tissue such as unresolved pneumonia, lung abscess, healing tuberculous cavities and infarcted areas of lung. In this form of infection a compact mass of fungous mycelium may develop and produce an aspergillus 'mycetoma' which is usually spherical in shape.

The other form of infection is an allergic type. The first account of this type of infection was by Hinson and colleagues (1952) who described three cases. In the opinion of these authors sensitisation by the fungus leads to the production of an exudate of fibrin, mycelium and mucus in the bronchial lumen which produces collapse and consolidation. A case of allergic aspergillosis was described by Mann and Pasha (1959). The patient was an asthmatic boy who during the course of the illness developed Schonlein-Henoch purpura. In the allergic form of the disease, cytological examination of the blood and sputum, shows the presence of eosinophilia.

The tumour like forms of aspergillosis were described by Villar and colleagues (1962). In this paper five cases were reported where the

fungus had invaded already diseased lung tissue and produced intra cavitary masses of mycelium. These authors who are Portuguese, remarked on the increasing number of such cases and reviewed twenty five other cases from Portuguese literature. In all these it was possible to trace a previous broncho-pulmonary disease on which the fungal infection had developed. In the opinion of these authors the fungus in the cavity does not behave as a realy pathogenic fungus, but produces a reaction similar to the presence of a foreign body. A few cases of this type of disease have been described where the infection was considered to be primary (Braatelieu and Perlmutter, 1961; Bruce, 1957; Hinson and colleagues, 1952; Vellios and associates, 1957). The last authors considered that the fungus ball developed within a previously dilated bronchus or bronchial cyst, and it was unlikely this would occur where the lumen of the bronchus was normal.

The lesion in pulmonary aspergillosis may not be localised to a mycelial mass but be more diffuse in distribution. Cases of this nature occurring as a complication of pneumonia have been reported (Abbott and colleagues, 1952; Darke and associates, 1957; Bech, 1961; Braatelieu and Perlmutter, 1961). A case of this type where the

infection was primary was described by Stevenson and Reid (1957).

Secondary infection of the pleural space may occur and such cases were reported by Hiddlestone and colleagues.(1954) and Manning and Robertson (1959).

As it is apparent that pulmonary aspergillosis is a condition which has become more common in recent years, it is relevant to consider the nature of the infecting agent and the conditions which predispose to invasion by the fungus.

Evidence of the ubiquitous nature of this fungus is seen in the ease with which it contaminates laboratory cultures. Microscopically all aspergilli are characterised by conidospores expanding into large vesicles at the end. From this vesicle shorter stalks arise into the shape of bristles of a brush, each stalk bearing a row of spores which by their colour give the tints of the culture. These spores are the cause of laboratory contamination and infection of the lung tissue. There are four species which are pathogenic to man - A. fumigatus, A. nidulans, A. niger, and A. versicolor. The first is the type most frequently isolated.

The sources of infection are many and varied and include birds, grain, flour, and coal. The literature on the subject is reviewed by Bruce

(1957). Primary infection may occur where there is exposure to material heavily contaminated with spores. In the cases reported by Hinson and colleagues (1952) there was contact with grain and flour. There was a history of exposure to grain dust in two primary cases described by Braatelieu and Perlmutter (1961) and cotton dust was considered to be the source of infection of the case reported by Stevenson and Reid (1957).

More commonly the infection is of a secondary nature and it would appear that this form of infection has become more prominant with the widespread use of antibiotic chemotherapy in the treatment of bacterial infection of the lungs. It is considered these drugs suppress the growth of bacteria thereby leaving the tissue open to infection by fungi. Previous antibiotic treatment was considered to be the predisposing factor by several workers (Hiddlestone and colleagues, 1954; Abbott and colleagues, 1952; Darke and associates, 1957; Manning and Robertson, 1959; Bech, 1961; Braatelieu and Perlmutter 1961). It was considered by Bech (1961) that the antibiotic therapy stimulated the growth of the fungus and probably its virulence. Riddell (1958) expressed the opinion that once infection is established,

changes occur in the adjacent tissues which promote further invasion by the fungus, and that pathogenicity was related to special products of fungal metabolism.

A diagnosis of aspergillosis rests on the findings of both the clinician and the bacteriologist. As the fungus may vegetate in the lung, a single isolation of Aspergillus from the sputum does not determine the presence of infection. The necessity of numerous examinations of the sputum was stressed by Riddell (1961). Stevenson and Reid (1957) considered a diagnosis could only be made by recovery of the fungus from a specimen obtained by bronchoscopy.

Culture of Aspergillus does not present difficulties as it grows readily on blood agar and Sabouraud's agar. This property of rapid growth may obscure the diagnosis as it may hide or overgrow slower growing fungi which may be the true cause of the infection. (Manual of Mycology 1944). Hinson and colleagues (1952) found blood agar adequate for culture and considered incubation at 45°C suitable as this tended to produce purer cultures.

Treatment of pulmonary aspergillosis may depend on the type of lesion. Where the condition is localised as in a mycetoma, surgical

excision may be performed. This was the form of treatment recommended by Villar and colleagues (1962) where the general condition of the patient permitted surgical intervention. In cases where surgical treatment is inadvisable or where the lesion is not localised, the local administration of anti fungal drugs may be effective in suppressing the Aspergillus. Riddell (1956) considered nystatin, suspensions of brilliant green, and hydroxystilbamidine were of value in the treatment of aspergillosis.

MONILIASIS

The genus Monilia consists of fungi which predominate in a unicellular form and which elongate to form filaments on submerged growth. M. albicans is the species which is important as a potential human pathogen.

The fungus is a normal inhabitant of the upper respiratory tract, the skin and the intestinal tract, and infection is of an endogenous nature. Because of its saprophytic nature, difficulties may arise in assessing the significance of isolation of the fungus from the sputum.

There have been several studies as to the incidence of Monilia in the sputum. In a review of the literature by Skinner (1947), the

percentage rate of isolation of Monilia ranged from 8.5% to 75%. Armstrong and Hall (1956) found an incidence of Monilia in routine specimens of sputum of 63.5%. It was considered by Scott Paul (1958) that M. albicans was present in the sputum of 30% to 40% of the population. A diagnosis of moniliasis of the respiratory tract should not be made lightly as the isolation of Monilia from the sputum does not in itself warrant a diagnosis of fungal infection. Scott Paul (1958) considered that this could only be made conclusively by lung biopsy and the demonstration of Monilia in the lung tissue. The doubtful significance of a single culture of Monilia was stressed by Conn and associates (1959). In the opinion of Skinner (1947) a diagnosis of moniliasis of the respiratory tract should be made only when all other diseases have been excluded. The importance of the exclusion of tuberculosis was stressed by Cohen (1953) and Drouhet (1957). This last worker considered that only histological criteria would definitely prove a broncho pulmonary moniliasis. In a Manual of Clinical Mycology (1944) it was stated that the diagnosis of respiratory monilial infection could be made with reasonable certainty only when M. albicans was coughed up constantly and in large numbers.

Several writers have reported cases of pulmonary moniliasis (Wiese and Bixby, 1941; Bass and Macfarlane, 1954; Wolff, 1952; Browne, 1954; Falkner and Wising, 1955).

The reason for the occasional assumption of a pathogenic role by M. albicans remains undiscovered.

It has been postulated that antibiotics stimulate the growth of fungi (Zimmerman, 1950; Huppert and Cazin, 1955). Evidence disproving this theory has been produced (Vieu, 1955; Marselou and Sergretain, 1954; Robinson, 1954). Interference with the synthesis and absorption of the vitamin-B complex following antibiotic treatment with a resultant lowering of resistance to invasion by the fungus was considered a possible explanation by Zimmerman (1950) and Harris (1950). Meenan (1957) however considered that the administration of vitamin-B did not affect the incidence of monilial infections in patients receiving antibiotic treatment.

As all species of Monilia appear similar in a wet or stained film, identification of M. albicans depends on a study of cultural characteristics and biochemical reactions.

Castellani (1927) was an early investigator who used almost exclusively the fermentative

properties of sugars for the identification of the species of Monilia. In 1931 Benham described the growth of chlamdospores in corn meal agar and expressed the opinion that the presence of these spores was a characteristic feature of M. albicans. Later writers expressed agreement with these findings (Martin and colleagues, 1937; Martin and Jones, 1940; Skinner, 1947).

Where the possibility of monilial infection arises it is desirable to identify the infecting species. It would appear advisable to include sugar fermentation as this would be a means of identification of the less commonly found species of Monilia which occasionally assume a pathogenic role.

FARMER'S LUNG

This is an occupational disease occurring mainly among hay and grain workers. The first description of the disease was by Campbell in 1932, who observed its appearance after a wet summer, and it would appear that such climatic conditions may increase the incidence of the illness. When crops which have been gathered in damp weather are handled, large quantities of dust containing vegetable matter and spores are produced. It is the inhalation of this dust which leads to the onset of the disease. There

is extreme dyspnoea and a dry iritant cough; the condition resolves spontaneously but re exposure to the dust will lead to a recurrence of the symptoms.

Fawcitt (1936,1938) concluded from the culture of similar moulds from the sputum and hay dust, that this condition was a fungus infection of the bronchi and lungs. The fungi which were considered to be of possible significance included Aspergillus, Penicillium, and Mucor. The view that the condition was the result of infection by the spores of ubiquitous fungi was held until more recent years.

Fuller (1953) considered the condition was the result of sensitisation to the fungus spores. In this study culture of the sputum yielded several fungi but the findings were not constant. No fungi were isolated from three of four specimens removed at bronchoscopic examination. Studdert (1953) regarded the condition as a non specific interstitial lung reaction to some material in the fungus rather than a true fungus infection of the lungs. Exposure to mouldy hay dust produced an excerbation of symptoms in one patient studied by Balders and Peter (1960). Lung biopsy by these authors showed the condition to be a focal non-caseating granuloma surrounded by giant cells and

accompanied by interstitial lymphocytic and histiocytic infiltration. In a study of workers exposed to the spores of Aspergillus and Penicillium, Horejsi and colleagues (1960) observed that directly after exposure, most subjects suffered from a condition resembling farmer's lung. Skin and serological tests showed sensitisation to these fungi. There was no evidence of mycetoma in any cases, but it was found there was an increased incidence of chronic bronchitis among the workers studied.

SPUTUM

As a bacteriological investigation of respiratory disease must be largely concerned with the examination of sputum, it is relevant to consider this secretion with regard to the distribution of bacteria and the cellular content.

Bacteriological examination of the sputum is a convenient and generally satisfactory method of estimating the degree of infection in the respiratory tract. It is however of great importance that the specimen should be a true discharge from the bronchial tree, as saliva, or saliva contaminated sputum may give rise to misleading results. Mulder (1956) commented on the advantage of a preliminary Gram film which

will reveal inadequate washing of the specimen. One difficulty encountered in the bacteriological examination of sputum is contamination by the bacterial flora of the mouth and pharynx. Straker and colleagues (1939) gave the carrier rate for pneumococci in the naso-pharynx as 20% to 40% and for Haemophilus influenzae as 40 to 80%. Brumfitt and colleagues (1957) devised a method of swabbing the bronchi, and compared the results of culture of these swabs with sputum culture. In forty two cases where the bronchial tree was sterile, pathogenic organisms particularly pneumococci were isolated from the sputum and throat swabs on twenty four occasions. There were also twenty seven cases with various bronchial lesions. In these cases it was found there was a higher isolation rate of pathogens particularly pneumococci from the sputum and throat swabs than from the bronchial swab. It was considered that where a pathogen was isolated from both sputum and throat swab, the sputum was not infected but contaminated by flora from the naso pharynx. In the opinion of these authors, examination of both throat swabs and sputum was of value as where the pathogen was present in the sputum only, it could then be regarded as having originated from the bronchial tree. In 1958 Brumfitt and Willoughby examined

the sputum and throat swabs of 117 patients with chronic respiratory disease. In about half the cases the same pathogenic organisms were present in both specimens but the incidence of pneumococci in the throat was greater than that of Haemophilus influenzae. Typing of the pneumococci was not done in either of these studies. This investigation would have provided additional information on the possible significance of the strains isolated from the various sources. Pecora and Yegrain (1958) compared the bacteriology of tissues exposed at thoractomy with expectorations and bronchial aspirations. It was found that the bronchi are sterile not only in healthy people but also frequently in patients with chronic diseases of the lower respiratory tract. Benstead (1950) examined bronchial secretions obtained by bronchoscope and so uncontaminated by mouth flora. Haemophilus influenzae was isolated from twelve and pneumococci from nine of the thirty cases of bronchiectasis in this study. There was a complete absence of pneumococci from cases of delayed pneumonic resolution suggesting a possible secondary infection by cocci of low grade pathogenicity. Anaerobic culture was also undertaken in this investigation. It was observed that the anaerobic cocci which were isolated, were

obtained more frequently from parenchymatous infections than from cases where the disease was mainly bronchial.

The bacteriologist studying respiratory infections does not, as a rule, have access to this type of specimen and has to rely on an examination of the secretion which the patient expectorates which, as has already been discussed, may vary in type. The type of illness suffered by the patient may have a bearing on this, for example where pain may prevent the patient coughing freely. In such circumstances examination of a laryngeal swab may be substituted. It is advisable that there is a close liason between the clinical and the laboratory staff, in order that the most suitable specimen is produced for examination.

The large variety of organisms isolated from the sputum in chronic bronchitis has been mentioned by Marshal (1931) and Southwell (1946). Howell (1951) demonstrated that the predominant organism may vary considerably over comparatively short periods of time. This finding prompted May (1952 1953a) to investigate more fully these variations. He considered that they might be due to irregular distribution of organisms in the sputum. He found that this was so and it appeared that the organisms most irregularly

distributed were those which most often appeared to be responsible for the infective element of the disease. Originally he cultured 30 parts of each specimen but found by using a random sampling technique the number could be reduced to five. Culturing several samples from one specimen of sputum is tedious and time consuming, and he advised the development of a technique of homogenising the sputum in order that only one culture need be made.

Such a method was developed by Rawlins (1953, 1955). To liquefy and homogenise the sputum he used 1% pancreatin in sterile physiological saline pH 7.6. He found that most sputa were liquefied in one hour or less. There was no interference with bacterial growth and a single culture from the liquefied sputum provided as reliable an index of the organisms present as five cultures from the unliquefied specimen.

In later studies of the bacteriology of the sputum in chronic bronchitis, May (1954 , 1956) used this technique of homogenising the specimens.

Digestion of the sputum was first described by Spengler (1894, 1895) who advocated its use in the isolation of tubercle bacilli. Later writers who have studied the digestive action of pancreatin on sputum and considered it

satisfactory in the treatment of sputum for the isolation of tubercle bacilli are Rice and Rowan (1953), Saxby (1954), and Saxholm (1955). The effect of the enzyme papain was investigated by Sullivan and Sears (1939) who found it an effective means of digesting the sputum for the isolation of tubercle bacilli.

In this study the effect of papain on organisms other than the tubercle bacilli was investigated. It was found that cultures of organisms including Haemolytic streptococci and pneumococci were unaffected after incubation in the enzyme for three hours.

Homogenisation of the sputum was produced by Elmes and colleagues (1953, 1955) by adding 5 ml. of physiological saline to 5 ml. of sputum and shaking for one hour. This was considered an effective method. Buchanan and associates (1958) homogenised the sputum by shaking the sputum with water and beads. This method was considered satisfactory and less time consuming than treatment of the specimen with pancreatin.

There is not a large number of statements on the chemical composition of sputum. The literature on the subject is reviewed by Basch and colleagues (1941).

In 1948 Sherry and co workers demonstrated

the presence of deoxyribonucleoprotein in purulent pleural exudates. This was the first identification of this substance as a significant constituent of purulent and inflammatory exudates and indicated its importance as a cause of thickness, viscosity and stringiness in pus.

Tillet and colleagues (1950) demonstrated that deoxyribonucleoprotein could be liquified by streptodornase, a deoxyribonuclease present in concentrates from cultures, of streptococci particularly group A.

The effect of this enzyme on viscous purulent sputum was studied by Armstrong and White (1950). Cytological examination of such sputum showed that there was a fibrillary structure due to deoxyribonucleoprotein. It was considered that movement due to respiration, coughing and ciliary action could all play a part in forming fibrils from leucocytic nuclei. When the sputum was treated in vitro with deoxyribonuclease, it was found that viscosity was reduced. Inhalation of the enzyme produced clinical benefit to the patients. Elmes and White (1953) also studied the effect of deoxyribonuclease in the treatment of purulent bronchitis. It was found that the enzyme reduced the viscosity of purulent sputum but

had no effect on mucoid sputum. In the opinion of Grant (1958) the inhalation of proteolytic enzymes was not of practical use and not to be recommended.

For the bacteriologist however, such methods of liquefaction of the sputum are not of practicable application.

The work of Helm and his colleagues (1954) indicated the necessity of cytological examination of the sputum in order to distinguish between eosinophils and neutrophils. In infective asthma, so called because of the macroscopic appearance of the sputum it was found that pus consisted mainly of eosinophils, and no pathogenic organisms could be isolated. May (1954) in a discussion of the indiscriminate use of antibiotics, stated that a cytological examination of the sputum should be undertaken before therapy was commenced, as sputum in which the 'pus' is composed of eosinophils would not respond to treatment with antibiotics. In this paper he showed that once eosinophils have been excluded as a cause of purulence of the sputum, pathogens can be isolated from all purulent sputum from cases of chronic bronchitis.

Rawlins (1955) investigated methods for a satisfactory cytological examination of the sputum. He considered that examination of a

single smear was not of value and advised homogenisation of the specimen with pancreatin. After homogenisation of the sputum, he counted the total number of cells per unit volume of sputum by means of a Neubauer Leucocytometer, but he did not consider such a reading of much value. Of greater assistance was a differential count. He considered that the staining effect of haematoxylin and eosin was superior to that of Leishman, one of the principle reasons being that it was an aid to the identification of degenerate eosinophils in the sputum.

In a study of the treatment of chronic asthma with prednisolone, Brown (1958) developed a technique for the identification of eosinophils which he considered time saving and effective. In this method the sputum was placed on a slide when a drop of Leishman stain followed by a drop of distilled water was applied to the specimen which was examined from ten minutes to several hours later. The advantage of such a method, over examination of a fixed smear, particularly in regard to speediness, would appear to be uncertain. Mulder (1956) also advocated the examination of a wet preparation of sputum. In this method the sputum was stained with eosin which coloured the eosinophilic granules dark red.

In view of the various reports of methods

identification of eosinophils, and of the value of the recognition of this type of cell in the sputum, it would appear that study of staining methods would be advantageous.

SCOPE OF THE PRESENT THESIS

The original studies embodied in this thesis are divisable into two parts. In the first part is a description of methods which were investigated with the object of finding the optimum conditions for the isolation of pathogenic organisms from the sputum. In the first place the effect of various homogenising agents on the sputum was studied, and secondly the most suitable culture media and environmental conditions for the isolation of pathogenic bacteria and fungi were investigated.

The second part of the thesis is a record of the application of the findings of the first part to the practical problem of the bacteriological examination of (a) 31 cases of chronic bronchitis and (b) 153 cases of pneumonia.

PART I

Experiment comparing the homogenising effect on sputum of water, saline, beads, and pancreatin

Material and methods

One hundred samples of sputum were examined to determine which of the following methods described below was the most satisfactory in producing liquefaction and homogenisation.

Each specimen was first classified according to viscosity and appearance. Degrees of viscosity were designated fluid, semi-fluid, viscid or very viscid. This was determined by tilting each specimen backwards and forwards in its container. The appearance was described as mucoid, muco-purulent or purulent. This was decided by naked eye inspection.

The samples of sputum were divided into four parts as nearly identical as possible and placed in universal containers. To the first were added four glass beads; to the second an equal quantity of sterile distilled water; to the third an equal quantity of normal saline (0.9%); to the fourth an equal quantity of pancreatin solution. This was prepared by adding one tablet of pancreatin (Oxoid) to 50 ml. of sterile distilled water. The tablet contains pancreatin 100 parts, sodium chloride 85 parts and phosphate buffer 12.8 parts. The final pH of the solution is 7.5. The mixture of pancreatin and sputum was shaken for ten minutes on a Kahn

shaker and then placed in a water bath for 90 minutes at 37°C. During this period at half an hour and one hour it was submitted to further shakings of ten minutes. All specimens with the exception of the one containing pancreatin, were placed on the shaker and remained there for 30 minutes.

After treatment, the degree of viscosity of the specimen was estimated by tilting the containers. The specimens were inspected in a strong light and the amount of homogenisation which had been produced was noted. A system of scoring was devised. No points were given if no change was observed in the specimen. One point was given where there was a moderate decrease in the viscosity and some homogenisation. Two points were given where there was a considerable decrease in viscosity and there was to the naked eye complete homogenisation of the sputum.

RESULTS

The results are set out in detail in Table I in the appendix and summarised in Tables 1, 2 and 3.

It can be seen that water, followed by pancreatin, were the most effective agents in producing homogenisation of the sputum. Table 1 shows the relationship of the type of sputum to the viscosity. The majority of the specimens were viscid (74%) and predominantly muco-purulent (47%). Tables 2 and 3 show the effect of the different methods of treatment in relation to the types of sputum and their degree of viscosity. It can be seen that water was most effective in liquefying and homogenising the sputum, (76% in table 2 and 70% in table 3).

The conclusion drawn from this study was that shaking with water was a satisfactory method of producing homogenisation of the sputum. Treatment with pancreatin was not so effective but it may have been that certain of the specimens did not receive adequate shaking. All the specimens were treated alike and did not receive individual attention.

In this study only the physical nature of homogenisation was considered. It was decided to investigate the bacteriology of sputum thus

treated, and also if the addition of beads to the water would further aid in the homogenisation of the specimen.

TABLE 1

(Summarised from Table I)

Relationship of appearance of sputum to degree of viscosity in 100 specimens

	Mucoid	Muco purulent	purulent	
Fluid	10	3	3	16
Semi fluid	3	1		4
Viscid	4	41	19	74
Very viscid		2	4	6
	27	47	26	100

TABLE 2

The effect of the homogenising agents in relation to the type of sputum

(Summarised from Table I)

The figures in black on the left of each column are the total scores obtained by each method for the particular type of sputum. The percentages in red are percentages of the total possible scores.

	Mucoid	Muco purulent	Purulent	Average percentage
Beads	13 24%	9 10%	7 13%	16%
Water	42 78%	68 72%	41 79%	76%
Saline	31 57%	50 53%	24 46%	52%
Pancreatin	34 62%	58 62%	33 63%	62%

TABLE 3

The effect of the homogenising agents in relation to the viscosity of the sputum.

(Summarised from Table I)

The figures in black on the left of each column are the total scores obtained by each method for the particular degree of viscosity of the sputum.

	Fluid	Semi fluid	Viscid	Very viscid	Average Percentage
Beads	17 53%	1 12%	11 7%	0 0%	18%
Water	30 94%	4 50%	107 71%	8 66%	70%
Saline	24 75%	3 37%	76 51%	2 17%	45%
Pancreatin	27 84%	3 37%	90 61%	5 42%	56%

The percentages in red are percentages of the total possible scores.

An experiment to ascertain if the addition of beads to sterile water was more efficacious in causing the homogenisation of sputum than the addition of water alone, and if culture of sputum homogenised by these methods was a more effective means of isolation of bacteria than culture of untreated sputum

Materials and methods

100 sputa sent to the laboratory for routine examination were studied.

They were classified according to appearance and viscosity. Appearance was graded as fluid, semi-fluid, viscid, and very viscid. The degree of viscosity was determined by tilting the specimen in the universal container. The macroscopic degree of purulence of the sputum was recorded as mucoid, muco-purulent, and purulent.

A blood agar plate was inoculated with the untreated specimen, the inoculum taken from a purulent part of the sputum. Two smears were also made at this time, taking as far as practicable the same portion of the sputum. The sputum was then divided into two equal portions. It was endeavoured to make these portions equal in appearance, consistency and amount. To the first was added an equal quantity of sterile distilled water with the addition of about six glass beads. The number of beads was not constant but varied to a small degree.

The specimens were then placed on the shaker and shaken until to the naked eye there was homogenisation and the specimen was fluid.

A blood agar plate was inoculated from each specimen and two smears made from each specimen.

The blood agar plates were incubated at 37°C for 18 hours when they were examined by means of a watchmaker's lens. All organisms which had grown were noted. There were two smears from each sputum. The first was stained Gram and the amount of pus and the organisms present observed. The second smear was stained Leishman and if the number of pus cells present was sufficient, a differential count was made.

Pneumococci were identified on the morphology and colonial appearance, solubility in 10% sodium desoxycholate, and sensitivity to optochin using a disk impregnated with a 1:4000 solution. Haemophilus influenzae was identified on the morphology and colonial appearance, and the reaction to Gram's stain. Staphylococcus aureus was identified by morphology and colonial appearance, and by coagulase production.

RESULTS

The length of time required for homogenisation of each specimen and the pathogenic organisms isolated can be seen in detail in Table II in the appendix.

When water alone was added to the sputum the period of time required to achieve homogenisation and liquefaction varied from 15 to 50 minutes. The average time of shaking was 34 minutes.

With the addition of beads to the water the time required ranged from 10 to 45 minutes. In this series the average time was 28 minutes.

Pathogenic organisms were isolated on culture from 47 specimens of sputum and the results have been analysed on Table 4. It can be seen that culture of sputum homogenised with water and beads, gave the highest isolation rate of pathogenic organisms, and was followed by culture of sputum homogenised by shaking with water only. In this experiment culture of untreated sputum was a much less efficient method of isolation of pathogenic organisms.

On Table 5 are the organisms which were present in the Gram films made from untreated sputum, sputum homogenised with water and beads, and water only. Here it was found that the same organisms were present in films made from

specimens homogenised by both methods. Fewer pathogens were seen in the Gram films made from untreated sputum than homogenised sputum, but the number of organisms seen in the films was greater than that found on culture of untreated sputum. This would indicate the advantage of a preliminary Gram film in bacteriological studies of the sputum.

By naked eye inspection of the sputum, 48 specimens were considered as mucopurulent or purulent.

In these 48 specimens, an examination of a Gram stained film of the untreated specimen, it was seen that in three specimens there was a very large number of pus cells, in eight specimens a moderate number of pus cells, in twenty seven a few pus cells, and in seven an occasional pus cell. It was possible in 35 specimens to do a differential count of the cells present, these films being stained by Leishman. Such counts revealed that over 90% of the cells present were polymorphs. There was no excess of eosinophils in any of the films under examination. When similar films were made after treatment of the sputum with water, and with water and beads, it was noted that although there were fewer cells present as a result of dilution, those cells which were

present were not in any way damaged and their identification presented no difficulty.

The results of this experiment show that homogenisation of the sputum with water and beads was more effective than with water alone. This superiority was evident in two ways. One was the shorter length of time required for homogenisation to take place, and the second was the larger number of pathogenic organisms isolated on culture.

This experiment also demonstrates that homogenisation of the sputum by water, and water and beads had no adverse effect on the bacterial and cellular content of the specimen. A higher isolation rate of pathogenic bacteria was obtained by culture of homogenised sputum than by culture of untreated sputum.

It was therefore decided to study more fully homogenisation of the sputum by this means.

TABLE 4

Pathogenic organisms isolated on culture from 47 specimens of sputum

(Summparised from Table II)

Pathogens isolated from sputum homogenised by shaking with water and beads.

Staphylococcus aureus	isolated from	21	specimens
Staphylococcus aureus and Haemophilus influenzae	" "	1	specimen
Staphylococcus aureus and Pneumococci	" "	1	specimen
Pneumococci	" "	21	specimens
Haemophilus influenzae	" "	3	specimens

Pathogens isolated from sputum homogenised by shaking with water only

Staphylococcus aureus	isolated from	20	specimens
Staphylococcus aureus and Haemophilus influenzae	" "	1	specimen
Pneumococci	" "	20	specimens
Haemophilus influenzae	" "	1	specimen

Pathogens isolated from untreated sputum

Staphylococcus aureus	isolated from	16	specimens
Staphylococcus aureus and Haemophilus influenzae	" "	1	specimen
Pneumococci	" "	18	specimens
Haemophilus influenzae	" "	1	specimen

TABLE 5

Pathogenic organisms present in Gram film of 47 specimens of sputum

(Summarised from Table II)

Pathogens present in sputum homogenised by shaking with water and beads

Staphylococci	present in	21	specimens
Staphylococci and Haemophilus influenzae	"	1	specimen
Staphylococci and Pneumococci	"	1	specimen
Pneumococci	"	21	specimens
Haemophilus influenzae	"	3	specimens

Pathogens present in sputum homogenised by shaking with water alone

Staphylococci	present in	21	specimens
Staphylococci and Haemophilus influenzae	"	1	specimen
Staphylococci and Pneumococci	"	1	specimen
Pneumococci	"	20	specimens
Haemophilus influenzae	"	1	specimen

Pathogens present in untreated specimens of sputum

Staphylococci	present in	18	specimens
Staphylococci and Haemophilus influenzae	"	1	specimen
Staphylococci and Pneumococci	"	1	specimen
Pneumococci	"	19	specimens
Haemophilus influenzae	"	1	specimen

A study of the bacteriology of the sputum comparing untreated sputum, sputum homogenised by shaking with water and beads, and sputum homogenised with pancreatin

Material and methods

In this experiment a series of 200 sputa were examined. These specimens had been sent to the laboratory to be examined for the presence of pathogenic organisms other than tubercle bacilli.

As 22 specimens were insufficient in quantity, homogenisation with pancreatin was omitted. In these specimens the examination undertaken was (1) examination of the untreated sputum, and (2) examination of sputum after homogenisation with water and beads.

178 sputum specimens were examined as follows:-

(1) A blood agar plate was inoculated with a purulent part of the sputum and a smear made of a similar portion.

(2) The sputum was then divided into two equal portions in universal containers. Into one was poured an equal amount of sterile distilled water and some glass beads. The number of beads was not constant and varied from 6 - 12, a larger number being used for a more thick and viscid specimen. This specimen was shaken for one hour on a Kahn shaker.

An equal amount of pancreatin solution (oxoid brand) was poured into the second half of the sputum. This was shaken for 10 minutes on the Kahn shaker, and placed in the water bath at 37°C for 50 minutes.

Blood agar plates were inoculated from each homogenised specimen. Smears were made and stained by Gram's method. The blood agar plates were incubated at 37°C for 18 hours.

Pneumococci were identified on the morphology and colonial appearance, solubility in 10% sodium desoxycholate, and sensitivity to optochin using a disk impregnated with a 1:4000 solution. Haemophilus influenzae was identified on the morphology and colonial appearance and the reaction to Gram's stain. Staphylococcus aureus was identified by morphology and colonial appearance, and by coagulase production.

RESULTS

Pathogenic organisms were isolated from 91 of the 200 specimens of sputum. Details of the organisms have been recorded on Table 6.

Staphylococcus aureus was isolated from 83 sputa. In 70 specimens it was the only pathogen isolated. On 9 occasions it was isolated together with Haemophilus influenzae, and with pneumococci on 4 occasions. In all, Haemophilus influenzae was isolated from 10 specimens and pneumococci from 11 specimens. Haemophilus influenzae and pneumococci were not present together in any of the specimens.

In Table 7 the organisms isolated from untreated sputum and homogenised sputum are recorded.

It can be seen that a higher isolation rate of Staphylococcus aureus was obtained on culture of the homogenised specimens. There was no difference in the isolation rate of pneumococci and Haemophilus influenzae from the three types of specimens.

Four degrees of growth were recognised on examination of the plates. These were, a few colonies = ±, a scanty growth = +, a moderate growth = ++, and a profuse growth = +++.

It was decided to give these degrees of

growth a system of scoring in order that a comparison could be made between the three examinations of the sputum.

Thus ± = ½ mark + = 1 mark ++ = 2 marks and +++ = 3 marks.

From the results of this system of scoring recorded on Table 8, it can be seen that there was a more profuse growth of organisms after homogenisation of the specimen.

As there were 22 fewer specimens treated with pancreatin, it was not possible to give a direct comparison. It did appear however that the results obtained by pancreatin homogenisation and water homogenisation were very similar.

The results of this experiment show that homogenisation of the sputum improved the isolation rate of Staphylococcus aureus. Although pneumococci and Haemophilus influenzae were isolated from the untreated specimen and the homogenised one with equal frequency, the degree of growth from the latter type of specimen was greater. The isolation rate of Haemophilus influenzae and pneumococci in this experiment was low. This could be due to omission of the use of selective cultural conditions for the isolation of these organisms.

TABLE 6

Pathogenic organisms isolated from ninety one specimens of sputum

Staphylococcus aureus	*Staphylococcus aureus* and *Haemophilus influenzae*	*Haemophilus influenzae*	*Staphylococcus aureus* and Pneumococci	Pneumococci
70	9	1	4	7

TABLE 7

Number of pathogens isolated from sputum before and after homogenisation

Pathogen	Untreated sputum	Sputum homogenised with water and beads	Sputum homogenised with pancreatin
Staphylococcus aureus	72	83	11 72 specimens not treated
Pneumococci	11	11	11
Haemophilus influenzae	10	10	4 6 specimens not treated

TABLE 8

Degree of growth of pathogens before and after homogenisation

Pathogen	Untreated sputum	Sputum homogenised with water and beads	Sputum homogenised with pancreatin
Staphylococcus aureus	91½ marks	156½ marks	(72 specimens) 126 marks
Pneumococci	16 marks	22½ marks	20 marks
Haemophilus influenzae	14 marks	16½ marks	(5 specimens) 9½ marks

½ mark = a few colonies; 1 mark = a scanty growth; 2 marks = a moderate growth; 3 marks = a profuse growth.

A bacteriological study of random samples of untreated and homogenised sputum

In this experiment specimens of sputum were homogenised with pancreatin, and by water and beads. By the bacteriological examination of five random samples from both types of homogenised specimens it was intended to find out if pathogenic organisms were distributed throughout the specimens.

Five random samples of the sputum before treatment were also examined in order to compare the isolation rate of pathogenic organisms from untreated sputum and homogenised sputum.

Materials and Methods

Fifty specimens of sputum were examined. These were received at the laboratory for examination for pathogenic organisms other than tubercle bacilli.

A loopful of the sputum was inoculated on blood agar, and Gram and Leishman films were made.

The sputum was then rinsed in physiological saline to remove adherent saliva and placed on a sterile Petri dish under which was placed a ruled grid of numbered squares. By random selection five specimens were taken from the sputum and inoculated on blood agar and Gram and Leishman

films made. The random numbers were taken from Fisher and Yates (1948).

The remaining sputum was transferred in equal parts to two sterile universal containers. To the first was added an equal quantity of sterile distilled water and glass beads. To the second was added an equal quantity of pancreatin solution prepared from 'Oxoid' pancreatin tablets.

The specimen containing the water was placed on the shaker and agitated until it appeared homogenised. The other specimen containing pancreatin was placed on the shaker for five minutes after which it was incubated in the water bath at 37°C for thirty minutes when it was again shaken for five minutes. If it appeared homogenisation had not taken place it was replaced in the water bath.

When homogenisation of the specimens had taken place five random samples were taken from each. Blood agar plates were inoculated with each sample and Gram and Leishman films were made.

The plates were incubated for eighteen hours at 37°C. Pneumococci were identified on the morphology and colonial appearance, solubility in 10% sodium desoxycholate, and sensitivity to optochin using a disk impregnated with a 1:4000

solution. Haemophilus influenzae was identified on the morphology and colonial appearance, and the reaction to Gram's stain. Staphylococcus aureus was identified by morphology and colonial appearance and by coagulase production.

RESULTS

The results are set out in detail in Table III in the appendix.

Numbers 1 to 2 denote specimens of sputum which have not been homogenised. Number 1 was taken direct from the universal container in which the sputum is sent to the laboratory. Number 2a to 2e were five specimens chosen at random by means of random numbers. Before these specimens were taken the sputum had been washed with saline to remove saliva and spread on a Petri dish. Numbers 3a to 3e were five specimens taken after homogenisation with water and beads had been completed. Numbers 4a to 4e were five specimens taken after homogenisation with pancreatin. These last ten specimens were all chosen by random, using the same technique as with the five untreated specimens, numbers 2a to 2e. There were thus 16 samples from each original specimen of sputum.

Pathogenic organisms isolated on culture

Pathogenic organisms were isolated from thirty five specimens.

These were:-

Staphylococcus aureus	from 13 specimens
Pneumococci	from 20 specimens
Haemophilus influenzae	from 1 specimen
Pneumococci and Haemophilus influenzae	from 1 specimen

These organisms were isolated on culture from all the samples of sputum homogenised with pancreatin, and water and beads.

A total of six samples were examined from each specimen of untreated sputum. There was a failure to isolate pneumococci from one or more samples of ten specimens of sputum

From one sample - nine occasions
From two samples - one occasion

There was a failure to isolate Staphylococcus aureus from eight specimens of untreated sputum.

From one sample - four occasions
From two samples - one occasion
From four samples - one occasion
From five samples - two occasions

Haemophilus influenzae was obtained from all samples of untreated sputum. In the specimen of sputum which contained pneumococci and Haemophilus influenzae, Haemophilus influenzae was absent from two of the random samples.

Examination of Gram film:

Pathogenic organisms present in all Gram films of sputum homogenised with pancreatin, and water and beads.

Staphylococci	13
Pneumococci	20
Haemophilus influenzae	1
Pneumococci and Haemophilus influenzae	1
	35

Pneumococci were not present in one or more of six samples from ten specimens of untreated sputum.

Absent from one	sample	in six	specimens
Absent from two	samples	in two	specimens
Absent from three	samples	in two	specimens

In five specimens of sputum pneumococci were obtained on culture from all the samples of untreated sputum but were absent from one or more of the Gram films.

In five specimens of sputum pneumococci were present in the Gram film of all samples of untreated sputum but were absent from one or more cultures of the untreated sputum.

Staphylococci were not present in one or more samples from seven specimens of untreated sputum.

Absent from one	sample	in three	specimens
Absent from two	samples	in one	specimen
Absent from three	samples	in two	specimens
Absent from four	samples	in one	specimen

In two specimens of sputum Staphylococcus aureus was obtained in culture of all the samples of untreated sputum but staphylococci were not present in one or more of the Gram films.

In three specimens of sputum staphylococci were present in the Gram film of samples of untreated sputum but were absent from one or more cultures of the untreated sputum.

In the specimen of sputum which contained pneumococci and Haemophilus influenzae, pneumococci were absent from three of the films of untreated sputum.

Examination of Leishman film

Sixteen films from each specimen were also stained with Leishman stain. This was in order that a differential count might be made of the cells present if there were sufficient. It was found that after treatment with water and with pancreatin there were not enough cells present for this purpose. This was probably caused by dilution of the specimen, but the cells which were present were not damaged either by the pancreatin or by shaking with beads and water. Their identification presented no difficulty. The majority of cells present were polymorphs, over 90% where a count was practicable, and in none of the specimens was there an excess of eosinophils.

Rate of homogenisation of sputum

When the sputum was homogenised and liquefied by shaking with water and beads, the time taken to achieve this varied from 10 minutes to 60 minutes, and the average time by this method was 33 minutes.

Using pancreatin as a homogenising agent, the time ranged from 15 minutes to 125 minutes, and the specimen which had been treated for 125 minutes was not adequately homogenised. The average time in this series was 60 minutes.

DISCUSSION

In this experiment fifty specimens of sputum were studied bacteriologically and pathogenic organisms were isolated from thirty five of these.

The results show that there was a failure to isolate pathogenic organisms from one or more of the samples of untreated sputum and this confirms the finding of May (1952, 1953a), that examination of a single part of the sputum may fail to reveal pathogenic organisms. To overcome this difficulty he recommended liquefaction of the sputum and in his later work employed pancreatin as the agent using the method of Rawlins (1953).

In the present study when pancreatin was used as a homogenising agent, pathogenic organisms were obtained on culture and in the Gram film from each of the five samples of the sputum selected by random.

Similar results were obtained when the sputum was homogenised with water and beads. This would indicate that when these methods are employed, the sputum is homogenised and the organisms distributed throughout the specimen.

Homogenisation with water and beads was a simpler technique than treatment of the specimen with pancreatin and as it appeared to give

equally satisfactory results it was decided to apply this method in the treatment of sputum.

As it was hoped in future studies of the sputum to include mouse inoculation it was considered that a specimen of sputum homogenised with water would serve two purposes, namely as an aid to a complete cultural examination and as a suitable specimen for animal inoculation.

An experiment to study the effect of homogenisation of the sputum with water, and water and beads on the deoxyribonucleo protein fibrils in the sputum

Materials and Methods

54 sputum specimens were examined. These particular samples were chosen by virtue of their purulence and viscosity as the purpose of the experiment was to investigate the fate of the D.N.P. fibrils present in such a viscous purulent sputum after the specimen had been shaken with an equal quantity of water and beads.

A smear was made from the untreated sputum which was then divided into two parts which were equal in volume, viscosity and purulence. The two portions were placed in universal containers and to each was added sterile distilled water equal in quantity to the sputum in the containers. To one of the specimens was also added about 6 glass beads. The two containers were then placed

on the Kahn shaker and shaken for 30 minutes. They were then examined in order to see what degree of homogenisation and lowering of viscosity had taken place. This was done by a naked eye inspection in a good light which revealed any small particles. The lowering of viscosity was tested by tilting the specimen in the container. If it was not considered that the specimen was adequately treated, it was replaced on the shaker. When it was finally decided that the specimen was both homogenised and liquefied, films were made from each sample so that in all three films were made from the one original specimen of sputum. These films were made with the same loop and distributed over the same area of slide.

It was decided to stain these films by the Feulgen reaction. This was preferred to the methyl green pyronin orange G stain which although shorter and simpler in technique does require a control slide with ribonuclease. This substance is very expensive 100 mg. costing £9. The method of using the Feulgen reaction was as follows:-

The three films were fixed with Susa solution for one hour and thereafter stained by the following method -

1. Rinsed in cold N/1 Hcl for one minute.

2. Hydrolysed in N/1 Hcl at 60°C for 15 minutes.
3. Removed and rinsed in N/1 Hcl for one minute.
4. Rinsed in water.
5. Stained in Feulgen solution (Schiff's reagent) for 2 hours.
6. Excess stain removed and slide placed in first of the staining jars containing sulphurous acid solution. Allowed to stand there for 10 minutes and the process repeated in the second and third jars.
7. Washed in water.
8. Counterstained in orange G for 3 minutes.

In stage 2 of the experiment, hydrolysis for 15 minutes gave the clearest differentiation between D.N.P. and the other constituents.

RESULTS

The results of the experiment are seen on Table IV in the appendix.

The following system of scoring has been adopted:-

A large number of pus cells	= 3 points
A moderate number of pus cells	= 2 points
A few pus cells	= 1 point
An occasional pus cell	= 0.5 points

When the D.N.P. fibrils were considered the degree of disitegration was estimated and scored as follows:-

Intact fibrils	= 3 points
Moderate degree of disintegration	= 2 points
Almost complete disintegration	= 1 point
Complete disintegration	= 0.5 point

It can be seen that water, and water and beads both reduced the number of pus cells. This may be due to dilution. The cells were intact and easily identifiable. The fibrils of D.N.P. were also much reduced in number and were always broken up to some degree. As far as the disintegration of D.N.P. fibrils was concerned there was little to choose between water, and water and beads. The scores were, water 32, and beads 30.5. However when beads were added to the water there was a considerable reduction in the time taken for homogenisation of the sputum. The average time for water was 41 minutes, while

for water and beads it was 33 minutes.

This experiment would indicate that homogenisation with water and beads is superior to the effect of water alone.

An investigation of staining methods for the identification of eosinophil polymorphs in the sputum

Materials and Methods

A short study was made with a view to determining how best eosinophils could be distinguished from other cells. Rawlins (1955) recommended haematoxylin and eosin, particularly as this stain was more satisfactory where the cells were degenerated. Leishman stain is however widely used and is simple in application. It was therefore decided to compare the two staining methods.

There are several varieties of haematoxylin and eosin and three methods were included in the survey. These were (1) Harris' alum haematoxylin (2) Ehrlich's alum haematoxylin (3) Mayer's acid alum haematoxylin.

Forty sputa were examined and the relative advantages of the three haematoxylin stains and and Leishman's stain were compared. Of the three haematoxylin stains it was decided that Mayer's method was the most satisfactory.

The final method decided upon was as follows.

Fix the film with methyl alcohol for thirty seconds. Apply the haematoxylin for ten minutes. Wash off stain with water and differentiate with 1% acid alcohol for fifteen seconds. Rinse and blue in tap water or tap water substitute. Counter stain with 0.5% eosin for fifteen seconds.

The method used for staining by Leishman method was as follows.

The undiluted stain was placed on the smear and remained for 2 minutes. The diluted stain was allowed to act for 5 to 7 minutes.

The results from this study indicated that staining with haematoxylin and eosin, and Leishman stain were equally effective methods of identification of eosinophils in the sputum.

Another series of sputa were studied to confirm this finding, and also to investigate if variations in the method of Leishman staining could be of advantage in the identification of eosinophils in the sputum.

In this second experiment 36 sputa were studied. The method of staining with haematoxylin and eosin has already been described.

It was found that the most effective demonstration of eosinophils by Leishman staining was obtained by applying the undiluted stain for two minutes and the stain diluted with distilled

water for ten minutes. The more prolonged staining rendered the eosinophilic granules more easily recognisable, particularly where the cells were degenerate.

This experiment confirmed that staining with haematoxylin and eosin and Leishman stain were equally effective methods of identification of eosinophils. As the technique required for Leishman stain was less time consuming than that required for haematoxylin and eosin, it was decided to employ it for future use.

An investigation into methods of identification of the pneumococcus

As the pneumococcus is an organism which is encountered frequently both in acute and chronic infections of the respiratory tract, it was decided to investigate fully the tests used in the identification of pneumococci. The reagents which it was considered of interestto investigate, were bile using rabbit bile, 10% sodium desoxycholate, 10% sodium taurocholate, 10% saponin and optochin. It was decided to study the effect of a disk of the substance placed on a blood plate inoculated with pneumococci, and also the tube solubility of the pneumococcus in bile, sodium desoxycholate, optochin, sodium taurocholate, and saponin. In addition it was felt that a study of the fermentative properties of the pneumococcus would be of value.

The carbohydrates which it was considered of interest to study were inulin, raffinose and mannite.

Materials and Methods

Out of a total of 208 strains obtained from cultures of specimens of sputum, 105 were considered to be pneumococci on colonial and morphological grounds, and the remaining 103 were strains of Streptococcus viridans.

Colonies for study were picked off and isolated in pure culture on blood plates, and used within 24 hours.

Rabbit bile, 10% sodium taurocholate, 10% sodium desoxycholate, 10% saponin, and 1:4000 solution of optochin (ethylhydrocuprein hydrochloride) were used in tubes and disks. The disks were prepared as for sensitivity tests according to the method of Gould and Bowie (1952). They were 6.25 mm. in diameter and were punched from No. 1 Whatman filter paper. Batches of 100 were placed in screw capped bottles and sterilised by dry heat at 150°C for 1 hour. The disks were then impregnated with the solution, 1 ml. of each being added to 100 disks. Each disk therefore containing 0.01 ml. of the solution.

One blood plate was inoculated from the pure culture by means of a loop to give an almost

confluent growth, and 5 ml . of 10% serum broth was also inoculated and incubated for 18 to 24 hours. Three blood plates were inoculated from this serum broth culture; the first with the undiluted culture, the second with culture diluted with an equal quantity of broth ; and the third with culture diluted 1 in 10 with serum broth. The technique of inoculation used was as for ordinary sensitivity tests, the plates being flooded with the broth and then allowed to drain on to blotting paper.

Five disks each impregnated with one of the above mentioned reagents were placed on each of the four inoculated plates which were then incubated at 37°C for 18 to 24 hours. The zone on inhibition measured with dividers was taken as the distance between the edge of the disk and the edge of the growth.

TUBE TESTS

For the tube tests, a 10% serum broth culture was used. One part of each of the four reagents, rabbit bile, 10% sodium taurocholate, 10% saponin and 1:4000 solution of optochin was added to 10 parts of serum broth culture. 0.1 part of 10% sodium desoxycholate was added to 5 ml. of serum broth culture. The tubes were incubated at 37°C observed occasionally for lysis

and the final reading was taken at 30 minutes.

Fermentation Reactions

Methods

Hiss's serum water was prepared by mixing 3 parts of 0.1% peptone water and 1 part of serum and to this solution was added Andrade's indicator. It was then divided into three portions and to each was added 1% inulin, 1% raffinose, and 1% mannite.

Each sugar medium was inoculated with 208 strains of organisms, 105 of which were pneumococci and 103 a strain of streptococci, probably Strept. viridans. After inoculation they were incubated for 48 hours.

RESULTS

Tube tests

The solubility of the pneumococci and Strept. viridans in saponin 10%, rabbit bile, sodium desoxycholate 10%, sodium taurocholate 10%, and optochin 1:4000 solution can be seen on Table 9. As saponin caused lysis in only one of the first 50 strains of pneumococci and Strept. viridans it was not tested further. 181 strains were tested with rabbit bile and 208 with the other reagents. The incomplete study with rabbit bile was due to a shortage of this substance. Optochin did not give satisfactory results. A few strains of pneumococci were soluble but generally the addition of this reagent caused cloudiness. Sodium desoxycholate was the most effective method of lysing pneumococci. 101 strains out of a total of 105 were soluble in this reagent. The next most effective method was the use of sodium taurocholate followed by rabbit bile. Only two strains of Strept. viridans were lysed, one in sodium desoxycholate, and the other in sodium taurocholate.

Disks

In Tables V and 10 and 11 are the results obtained by using disks impregnated with rabbit

bile, sodium desoxycholate, sodium taurocholate and optochin, using as inocula (a) a loopful of pneumococci from a blood agar plate, (b) broth cultures of pneumococci and Strept. viridans undiluted (c) broth cultures of pneumococci and Strept. viridans diluted 1:2 (d) broth cultures of pneumococci and Strept. viridans diluted 1:10.

In Tables V a,b,c, and d, in the appendix, the results are classified according to the degree of inhibition and the method of inoculating the plate. It can be seen that certain strains of pneumococci and Strept. viridans give equivocal results, i.e. pneumococci with no or very small zones of inhibition, and Strept. viridans with zones of inhibition of 4 mm. and over.

These results have been summarised in Tables 10 a,b,c, and d. Under equivocal have been grouped (1) pneumococci with no zone of inhibition, (2) pneumococci and Strept. viridans with zones of inhibition of 1-3mm (3) Strept. viridans with zones of inhibition of 4mm and upwards.

It can be seen that optochin gives the best results by all four methods of inoculation. That is to say the number of equivocal results is lowest and of definitive results highest with

optochin. The results given by sodium desoxycholate follow a close second, while sodium taurocholate and bile are about equal.

When the zone of inhibition produced by all four reagents is considered it can be seen from Table 10d that a 1:10 dilution of broth culture provides the best inoculum, because the number of equivocal results is lowest and of definitive results is highest. A broth culture of 1 in 2 is second but on the whole rather far behind; undiluted broth is even less satisfactory while inoculum from a culture on blood agar by means of a loop is not at all satisfactory and yields a high proportion of equivocal results.

The average size of zones is greatest with optochin (8mm.) while sodium desoxycholate again comes second with an average zone measurement of 5mm., and sodium taurocholate with an average zone measurement of 4.7mm., and bile with an average zone measurement of 5mm are about equal. These results are presented on Table 11.

Fermentation reactions

The results of the fermentation tests using inulin raffinose and mannite are given on Table 12. Inulin was fermented by 87 of 105 strains of Pneumococci, while 84 of 103 strains of Strept. viridans failed to ferment it. Raffinose was

fermented by 81% of strains of pneumococci and 96% of strains of Strept. viridans. Mannite was fermented by 14 strains of pneumococci and by 4 strains of Strept. viridans. From these results it would appear that inulin is the only useful differentiating agent.

Four strains of pneumococci were insoluble in sodium desoxycholate. Two of these were sensitive to optochin and all fermented inulin.

Discussion

When the various reagents causing lysis of the pneumococci are considered, it is apparent that sodium desoxycholate was the most effective. It was considered of value to test the reactions of bile, sodium taurocholate, and saponin as these reagents are all regarded as possessing the property of dissolving pneumococci.

Neufeld (1900) observed that pneumococci were soluble in bile. In 1907 Levy investigating the active principle of bile which caused solubility, found sodium taurocholate would dissolve pneumococci. Mair (1917,1929) and White (1929) recommended the use of sodium desoxycholate and considered its action superior to that of sodium taurocholate. The use of saponin as a lytic agent was recommended by Downie and colleagues (1931), and Klein and Stone (1931). In the present experiment saponin was not found to be an effective agent in causing lysis of pneumococci.

83% of the strains of pneumococci fermented inulin. In the opinion of Hiss (1902) and Hiss and colleagues (1906), inulin fermentation was a definite property of the pneumococcus.

From the findings of the present study where certain strains of pneumococci failed to ferment this sugar and 18% of the strains of Strept. viridans studied did so, it would be unwise to identify pneumococci on the results of this test only. It was considered by Topley and Wilson (1955) that probably all fresh strains of pneumococci ferment inulin but it is not advisable to regard fermentation of inulin as an infallible test.

When an inoculum consisting of a broth culture of pneumococci diluted 1:10 was used, it was found that almost all the strains were sensitive to the action of optochin. The use of optochin for identifying pneumococci was described by Bowers and Jeffries (1955) and Bowen and colleagues (1957). Masters and colleagues (1958) considered optochin sensitivity a satisfactory and simple way of identifying pneumococci.

As the properties of solubility in sodium desoxycholate, inulin fermentation, and optochin sensitivity were absent in some strains of the pneumococci studied in the present investigation, it was considered that for a complete identification of the pneumococcus all these properties should be investigated in future studies of the organism.

Table 9.

Solubility of pneumococci and Strept. viridans in saponin, Bile, Sodium taurocholate, Sodium desoxycholate and Optochin.

		Saponin	Bile	Sodium taurocholate	Sodium desoxycholate	Optochin
Pneumococci	Lysis	1	85	94	101	16
	No Lysis	28	7	11	4	89
Str. viridans	Lysis	0	0	1	1	0
	No Lysis	21	89	102	102	103

Tables 10a, b, c and d.

The size of zone of inhibition produced by disks impregnated with bile, sodium desoxy cholate, sodium taurocholate, and optochin on 105 strains of pneumococci and 103 strains of Strept. viridans using four methods of inoculation.

(A summary of tables Va, b, c and d)

Table 10a

Inoculum - Loop from blood agar plate

		Bile	Sodium taura cholate	Sodium desoxy cholate	Optochin
Zone of inhibition present	Pneumococci	64	53	82	91
Equivocal result		80	83	61	39
Zone of inhibition absent	Str. viridans	64	72	65	78

Table 10b.

Inoculum - Broth undiluted

		Bile	Sodium tauro cholate	Sodium desoxy cholate	Optochin
Zone of inhibition present	Pneumococci	56	45	66	77
Equivocal result		51	61	42	28
Zone of inhibition absent	Str. viridans	101	102	100	103

Table 10c

Inoculum - Broth diluted 1:2

		Bile	Sodium taurocholate	Sodium desoxycholate	Optochin
Zone of inhibition present	Pneumococci	71	72	89	94
Equivocal result		35	33	17	11
Zone of inhibition absent	Str. viridans	102	103	102	103

Table 10d

Inoculum - Broth diluted 1:10

		Bile	Sodium taurocholate	Sodium desoxycholate	Optochin
Zone of inhibition present	Pneumococci	91	83	99	98
Equivocal result		16	23	8	7
Zone of inhibition absent	Str. viridans	101	102	101	103

Table 11.

Average zone in m.m. obtained in the disk method with the four reagents and the four methods of inoculation.

	Loop	Broth undiluted	Broth diluted 1:2	Broth diluted 1:10
Bile	4.9	4.7	4.8	5.0
Sodium tauro cholate	3.6	4.3	4.6	4.7
Sodium desoxy cholate	5.3	5.8	5.5	6.5
Optochin	6.7	5.3	6.2	8.0

Results of Fermentation Reactions

Table 12.

	Inulin	Raffinose	Mannite	
Fermentation present	87	85	14	Pneumococci
Fermentation absent	18	20	91	
Fermentation present	19	99	4	*Str. viridans*
Fermentation absent	84	4	99	

Additional experiment on the fermentative properties of pneumococci.

It had been observed in previous experiments that certain strains of pneumococci did not possess the property of fermenting inulin. These experiments had all been performed under aerobic conditions of culture.

Austrian and Colowick (1953) studied the fermentation of inulin by pneumococci and was of the opinion that in the appropriate environmental conditions there were no strains which failed to ferment this sugar. They considered that the access of oxygen to the cells in the fermentative medium, might contribute to the formation and accumulation of peroxides which could result in a failure to produce acid.

It was therefore decided to study the fermentative action of pneumococci under anaerobic conditions.

The medium employed was a 1% solution of inulin in Hiss's serum broth with Andrade's indicator. To produce anaerobic conditions the tube was sealed with sterile vaseline. Two tubes of media were inoculated with each strain of pneumococcus, one tube being incubated under aerobic conditions and the other under anaerobic conditions.

Twenty strains of Strept. viridans were examined under the same conditions.

Results

Details of the results of the experiment can be seen on Table 13.

The fermentative reactions of fifty four strains of pneumococci were studied. Under anaerobic conditions of culture all these strains fermented inulin. Nine of these strains of pneumococci failed to ferment inulin when incubated under aerobic conditions. It was also observed that the length of time required for the production of inulin fermentation was shorter in eighteen of the strains when incubated under anaerobic conditions. In fourteen strains, fermentation took place twenty four hours earlier, and in four strains fermentation took place forty eight hours earlier under anaerobic as compared with aerobic conditions.

Twenty strains of Strept. viridans were studied under the same conditions. One strain fermented inulin both aerobically and anaerobically after eighteen hours incubation. No fermentation was observed with the remaining nineteen strains.

TABLE 13.

Aerobic culture	Period of time for fermentation
Inulin fermentation	Days
1. positive	3
2. negative	
3. positive	2
4. "	1
5. "	1
6. "	1
7. "	1
8. "	2
9. negative	
10. positive	2
11. "	1
12. "	2
13. "	1
14. "	2
15. "	1
16. "	1
17. "	1
18. negative	
19. positive	1
20. "	1
21. "	1
22. "	3
23. "	1
24. "	1
25. "	3
26. "	3
27. "	1
28. negative	
29. "	

Results Anaerobic culture	Period of time for fermentation
Inulin fermentation	Days
1. positive	2
2. "	2
3. "	1
4. "	1
5. "	1
6. "	1
7. "	1
8. "	2
9. "	1
10. "	1
11. "	1
12. "	1
13. "	1
14. "	2
15. "	1
16. "	1
17. "	1
18. "	2
19. "	1
20. "	1
21. "	1
22. "	1
23. "	1
24. "	1
25. "	2
26. "	1
27. "	1
28. "	1
29. "	1

TABLE 13

Results Aerobic culture	Period of time for fermentation
Inulin fermentation	Days
30. positive	3
31. "	3
32. negative	
33. positive	1
34. "	1
35. negative	
36. positive	2
37. "	2
38. "	2
39. "	2
40. "	2
41. "	2
42. negative	
43. positive	1
44. "	1
45. "	3
46. "	1
47. negative	
48. positive	1
49. "	1
50. "	1
51. "	1
52. "	1
53. "	2
54. "	1

TABLE 13 cont.

Results	Period of time for fermentation
Anaerobic culture	
Inulin fermentation	Days
30. positive	1
31. "	1
32. "	5
33. "	1
34. "	1
35. "	1
36. "	1
37. "	1
38. "	2
39. "	2
40. "	2
41. "	1
42. "	1
43. "	1
44. "	1
45. "	2
46. "	1
47. "	2
48. "	1
49. "	1
50. "	1
51. "	1
52. "	1
53. "	1
54. "	1

Discussion

From this experiment it would appear that anaerobic culture was more effective than aerobic culture in demonstrating the fermentation of inulin by pneumococci. Although all the strains of pneumococci in this study fermented inulin when incubated anaerobically it cannot be claimed that all strains of pneumococci would do so as the number of organisms examined in this experiment was too small to warrant such a conclusion.

It was also observed that fermentation of inulin was produced more rapidly in certain strains when in anaerobic conditions than in aerobic conditions. Such a faculty is of advantage in routine laboratory work.

The method used in this experiment to produce anaerobiosis was simple but appeared to be effective. It was considered to be the most practicable method for use in this type of experiment.

An investigation of methods of isolation of Haemophilus influenzae

The object of this investigation was to study the culture of sputum infected with Haemophilus influenzae on selective media. In addition to estimating the cultural advantages of the media, untreated sputum and sputum homogenised by water and beads were examined in order to see if a superior isolation rate of Haemophilus influenzae was obtained from the homogenised specimen.

Material and methods

33 specimens of sputum were examined.

The media investigated were

1. Heated blood agar
2. Levinthal's medium with and without penicillin.
3. Fildes medium with and without penicillin.

The amount of penicillin incorporated in the media was 0.5 units/ml.

A loopful of the purulent part of the sputum was inoculated on all plates and a Gram was made. The sputum was then homogenised by adding an equal amount of water and glass beads. The specimen was shaken for at least thirty minutes and for a longer period if this was considered necessary.

When the specimen was homogenised a loopful of the specimen was inoculated on all plates. The plates were incubated at 37°C for eighteen hours.

Results.

Culture media

A growth of Haemophilus influenzae was obtained on culture of the homogenised specimen on all culture media employed viz;
(1) heated blood agar (2) Levinthal's medium with and without penicillin (3) Fildes medium with and without penicillin.

A growth of Haemophilus influenzae from untreated sputum was obtained on 24 occasions from heated blood agar, on 25 occasions from Fildes medium with and without penicillin, on 26 occasions from Levinthal's medium with and without penicillin.

Gram film:

Untreated sputum

Haemophilus influenzae present in - 25 specimens

Homogenised sputum

Haemophilus influenzae present in - 33 specimens

Type of sputum examined:

Purulent - 22 specimens

Mucopurulent - 11 specimens

Discussion.

The number of Haemophilus influenzae in this experiment is not large but it was intended it should act as a guide in future work.

It was found that equally successful results were obtained with all the culture media employed and it was not considered that Levinthal or Fildes media were superior to heated blood agar in the isolation of Haemophilus influenzae. The addition of penicillin to the media did not appear to be an advantage. There was no marked overgrowth of other organisms in the plates without the penicillin.

When a comparison was made between the isolation rate of Haemophilus influenzae and from untreated sputum and homogenised sputum it was found that the latter gave better results.

Examination of a Gram film showed that Haemophilus influenzae was present in all the homogenised and in twenty five of the untreated specimens.

From the results of this experiment it was concluded that the most effective method of isolation of Haemophilus influenzae was the inoculation of homogenised sputum on a selective medium.

As equally good results were obtained with heated blood agar as with Levinthal and Fildes' media it was decided to employ this medium in future studies of Haemophilus influenzae. It was considered that heated blood agar was the most simple to produce.

An investigation of methods of identification of the species of Monilia

The object of this study was to develop a method of identification of Monilia which was uncomplicated yet comprehensive, and suitable for use in a routine laboratory. It is of course recognised that animal inoculation will indicate the pathogenicity of Monilia, but this examination was not practicable and purely laboratory investigations were undertaken.

Experiment 1a.

Material and methods

213 specimens of sputum were examined for the presence of Monilia. These specimens had been sent to the laboratory for examination for pathogenic organisms other than tubercle bacilli.

Blood agar and Sabouraud's agar were inoculated with the sputum and the cultures were incubated at 37°C. for three days. By this method of culture, 102 of the 213 specimens of sputum yielded a growth of a fungus morphologically resembling a Monilia.

In order to differentiate the species of Monilia, the following criteria were employed

1. Morphological appearance on blood agar and Sabouraud's agar.
2. Culture on corn meal agar.
3. Biochemical reactions of the following sugars (a) glucose; (b) lactose; (c) saccharose; (d) maltose; (e) raffinose; (f) galactose

Culture on corn meal agar

The corn meal agar was Oxoid brand. The fungi were picked from the Sabouraud plate with a straight wire and inoculated in the corn meal plate by cutting deeply into the medium. The corn meal plate was then incubated at 22°C for three days when it was examined and Gram films made of the growth. If no chlamydospores were seen, the plate was replaced in the incubator and examined again in another four days, after which time if no chlamydospores were seen it was discarded.

Biochemical reactions

A 1% solution of the sugar in peptone water was used. An inverted Durham tube was employed to detect gas formation. Duplicate sets of the sugars were inoculated. In one series the tubes were plugged with sterile vaseline to obtain anaerobiosis. This was omitted in the second set. The sugars were inoculated with a loopful of a suspension of the monilia in sterile water. They were incubated at 37 °C for 5 days.

Results

Number of specimens of sputa examined 213.

Cultures of Monilia isolated 102 (48%)

Chlamydospore formation

The number of specimens showing chlamydospore formation was 23.

Results of biochemical reactions

Number of cultures showing the biochemical reactions of M. albicans - 83.

These reactions were glucose, and maltose, acid and gas; saccharose and galatose, acid; lactose and raffinose no change.

Experiment 1b.

This experiment was undertaken to improve if possible the number of isolations of chlamydospores which was low in experiment 1a.

Materials and methods.

The specimens under examination were the 102 specimens of Monilia studied in experiment 1a. The method employed was that of McKenzie (1958) who used the technique of Lodder and Van Rij (1952). The yeast culture was subcultured and incubated at 22°C for 48 hours. It was then purified by streaking on a malt agar plate and incubated for three days at 22°C. After the colonies had begun to develope, a single

isolated colony was removed and transferred to a fresh malt agar slant. This was the stock culture and the corn meal agar plate was inoculated from this plate. As in the previous experiment it was inoculated by cutting with a straight wire.

Results

The number of specimens showing chlamydospore formation was 13.

Experiment 2

This experiment was undertaken to improve on the number of isolations of chlamydospores as it was considered that the number obtained in the previous experiments (1a and 1b) was too low.

Material and methods.

214 specimens of sputum were examined for the presence of Monilia. The sputum was inoculated on blood agar and Sabouraud's agar and incubated at 37°C for three days. By this method of culture 114 specimens of sputum yielded a culture of a fungus morphologically resembling Monilia.

In order to differentiate the species of Monilia, the following criteria were employed.

1. Morphological appearance on blood agar and Sabouraud's agar.
2. Culture on corn meal agar.
3. Biochemical reactions of glucose, lactose, saccharose, maltose, raffinose, and galactose.
4. Growth on Sabouraud's glucose broth.
5. Culture on corn meal agar.

Culture on corn meal agar.

The medium used was Oxoid brand. A colony from the original culture on Sabouraud's agar was inoculated on blood agar and incubated for three days at 37°C. The corn meal plate was inoculated with this culture by cutting the surface with a straight wire and incubated at 22°C for three days when it was examined for chlamydospores by study of a Gram film of the culture. This was repeated daily for the next four days.

Biochemical reactions

Two sets of sugars were inoculated, one aerobically and one anaerobically, the anaerobiosis being produced by plugging the tubes with sterile vaseline. The sugars were inoculated from the second Sabouraud plate, i.e. the subculture from the blood agar plate. The culture was suspended in sterile water and each tube inoculated with a loopful of the culture

and incubated for 5 days at 37°C when a reading was made. A 1% solution of sugar in peptone water was used with an inverted Durham tube to detect any gas formation.

Sabouraud's glucose broth was inoculated with the Monilia if the morphological appearance or the sugar reactions indicated that the fungus under examination might be one which produced a surface growth viz. Monilia tropicalis and Monilia Krusei.

Results

Number of specimens of sputa examined 214.
Cultures of Monilia isolated 114.

Chlamydospore formation

Number of specimens showing chlamydospore formation 97.

Results of biochemical reactions

Number of cultures showing the biochemical reactions attributed to Monilia albicans 89. These reactions were glucose and maltose, acid and gas; saccharose and galactose, acid; lactose and raffinose no change.

Of the 97 cultures where chlamydospore formation was present there were strains, 14, where the sugar reactions were not those of Monilia albicans.

Six cultures which resembled Monilia albicans morphologically and produced the biochemical reactions of that species, did not produce chlamydospores.

There were 11 monilias which were not Monilia albicans. The differential diagnosis was made by using the criteria recommended by Martin (1937) which are:- 1. growth on corn meal agar 2. sugar reactions 3. morphological appearance 4. growth in Sabouraud's broth.
They were identified as - 5 specimens of Monilia krusei, 1 of Monilia Stellatoidea, 2 of Monilia tropicalis and 3 strains which could not be identified.

DISCUSSION

In the preliminary stage of the experiments, the incidence of Monilia in the sputum was determined. Two separate sets of sputum specimens approximately equal in number were examined, and it was found that the isolation rate of Monilia from each group was very similar. Monilia was cultured from 48% of the first group and from 49% of the second group. It is not known how many of these specimens were received from patients who had received antibiotic treatment. None of the patients were investigated for a possible monilial infection and the presence of the fungus was not of importance in the patient's illness.

Moniliasis is generally caused by infection with M. albicans. The most typical characteristic of M. albicans is the production of chlamydospores when cultivated under suitable conditions. (Benham, 1931; Martin, 1937, 1940; Skinner, 1947).

In the first group of experiments (1a and 1b) the rate of chlamydospore formation was very low. It was considered that 23 out of 102 was not an accurate estimation of the incidence of M. albicans. In this investigation, 83 of the Monilia gave the sugar fermentative reactions considered typical of M. albicans. and of these only 20 showed the presence of chlamydospores. When the same

cultures were investigated by the method of Lodder and Van Rij as used by McKenzie (1958), the results were even poorer than before. Perhaps the age of the cultures had a detrimental effect, but it was considered that further investigation was required.

It was decided to subculture on blood agar in order to obtain a pure culture of M. albicans. It was possible that there was more than one type of Monilia on the Sabouraud plate and this would be undetected without subculture. By the use of blood agar certain species were identified e.g. M. stellatoidea by its typical star like appearance on this medium. From the results obtained in experiment 2 it appeared that the introduction of subcultivation on blood agar after initial isolation, improved the isolation rate of chlamydospores. It may be that by this method a more pure culture is obtained, and in addition the poorer conditions of growth may play a part for although Monilia can be cultured on blood agar, the growth is not so luxuriant as that obtained on Sabouraud's medium. Chlamydospores are more likely to grow where there is a poor condition of growth. (Benham 1931).

Castellani (1927) who was the pioneer in this century in the investigation of fungal infections,

placed great reliance on the fermentative properties of monilias as an aid to their identification. Later writers did not agree wholly with his views and considered that the other properties of the Monilia should be considered in the identification of the species. (Benham 1931).

However sugar fermentative tests are relatively simple to perform and in this series of experiments the results obtained were satisfactory. By the use of both corn meal agar and sugar fermentation tests, it was possible to increase the total number of strains of Monilia identified as M. albicans, as certain strains which did not produce chlamydospores were recognised from the sugar reactions.

In addition to the identification of M. albicans, a study of the biochemical reactions is of value in the identification of the less common types of Monilia which may be the occasional causes of infection. A further aid to the recognition of these rarer types, is culture on Sabouraud's glucose broth.

APPENDIX

TABLE I

The homogenising effect of beads, water, saline, and pancreatin on 100 specimens of sputum

Type		Visc-osity	Beads	Water	Saline	Pan creatin
1	mp	v	1	1	0	1
2	m	f	1	2	1	1
3	mp	vv	0	1	1	1
4	mp	v	0	1	0	0
5	mp	v	0	1	1	1
6	mp	vv	0	2	0	1
7	m	f	1	2	2	2
8	m	f	2	2	1	2
9	m	sf	0	2	1	1
10	m	f	1	2	1	2
11	m	v	0	1	0	0
12	mp	v	0	2	0	2
13	mp	v	0	2	1	2
14	mp	v	0	1	1	1
15	mp	v	0	2	2	2
16	p	v	1	2	1	1
17	m	f	1	2	2	2
18	p	v	1	2	1	1
19	mp	v	0	2	1	1
20	m	f	1	2	1	1
21	p	v	0	2	2	2
22	p	v	0	1	0	1
23	p	v	1	2	1	2
24	m	f	1	2	1	2
25	mp	v	0	2	1	2
26	p	vv	0	2	1	1
27	p	vv	0	1	0	0
28	m	sf	0	1	0	1
29	mp	v	0	2	2	1

TABLE I

Type		Visc-osity	Beads	Water	Saline	Pan creatin
30	mp	v	1	2	1	1
31	mp	v	1	2	2	2
32	m	f	1	2	2	2
33	p	v	0	2	2	2
34	p	v	0	2	1	2
35	m	f	1	2	1	2
36	mp	sf	0	1	1	0
37	m	f	1	2	1	1
38	mp	f	1	1	2	1
39	p	vv	0	1	0	1
40	mp	v	1	1	1	1
41	p	vv	0	1	0	1
42	p	v	0	1	1	1
43	m	v	0	1	1	1
44	mp	v	0	2	1	1
45	mp	v	0	2	1	1
46	p	v	0	0	1	1
47	p	v	0	2	0	1
48	mp	f	1	1	1	2
49	mp	v	0	1	1	1
50	p	v	0	2	1	1
51	mp	v	0	2	1	2
52	mp	v	1	2	1	1
53	p	f	1	2	2	1
54	p	f	1	2	2	2
55	p	f	1	2	1	1
56	p	v	1	1	1	1
57	mp	v	0	2	1	1
58	p	v	0	2	1	2
59	p	v	0	1	1	1

TABLE I

Type		Visc-osity	Beads	Water	Saline	Pan creatin
60	p	v	0	2	1	2
61	p	v	0	2	1	2
62	mp	v	0	1	1	1
63	mp	v	0	1	1	1
64	mp	v	0	1	1	1
65	mp	v	0	1	1	0
66	mp	v	0	1	1	2
67	mp	v	0	2	1	1
68	mp	sf	1	2	2	2
69	mp	v	0	2	1	1
70	mp	v	0	2	1	1
71	m	v	0	1	1	1
72	mp	v	0	2	1	1
73	mp	v	0	1	2	2
74	m	v	0	1	1	1
75	m	v	0	2	2	1
76	mp	v	0	1	1	1
77	m	v	0	1	1	1
78	mp	v	0	1	1	2
79	mp	v	0	2	1	1
80	m	v	0	2	1	1
81	p	v	0	1	1	1
82	mp	v	0	1	1	1
83	mp	v	0	1	1	1
84	p	v	0	1	0	1
85	mp	v	0	1	2	2
86	m	v	0	1	2	1
87	mp	v	0	2	1	1
88	m	f	1	2	2	2
89	m	v	0	1	1	1

TABLE I

	Type	Vis-cosity	Beads	Water	Saline	Pan creatin
90	mp	v	0	1	1	1
91	mp	v	0	1	1	1
92	m	v	0	1	1	1
93	m	v	1	2	1	1
94	mp	v	1	1	1	1
95	m	v	0	1	1	1
96	p	v	0	2	1	1
97	m	v	0	1	1	1
98	mp	v	0	1	1	1
99	m	v	0	1	1	1
100	mp	v	0	1	1	1
			29	151	105	125

mp - mucopurulent
p - purulent
m - mucoid
v - viscid
vv - very viscid
f - fluid
sf - semifluid

0 - No change observed in specimen.
1 - A moderate decrease in viscosity and some homogenisation
2 - A considerable decrease in viscosity and to the naked eye complete homogenisation of the sputum.

TABLE II

In this table is a record of the pathogenic organisms isolated on culture and present in the Gram film of untreated sputum, sputum homogenised by water, and sputum homogenised by water and beads, also the length of time taken by these reagents to produce homogenisation of the specimen.

	Time		Type		Culture			Gram Film		
	Water	Water and Beads			Direct	Water	Water and Beads	Direct	Water	Water and Beads
1	30 mins	25 mins	m	v						
2	35 "	25 "	p	v						
3	35 "	30 "	mp	v						
4	45 "	40 "	p	v						
5	20 "	15 "	m	v						
6	30 "	25 "	m	p						
7	30 "	25 "	m	v						
8	30 "	25 "	m	v						
9	40 "	35 "	p	vv						
10	20 "	15 "	m	sf						
11	40 "	30 "	p	v						
12	40 "	30 "	p	vv						
13	20 "	15 "	m	sf						
14	40 "	35 "	mp	vv						
15	40 "	35 "	m	sf						
16	30 "	25 "	m	sf						
17	40 "	35 "	p	vv						
18	30 "	25 "	mp	v						

TABLE II

	TIME		Type		CULTURE			Gram film		
	Water	Water and Beads			Direct	Water	Water and Beads	Direct	Water	Water and Beads
19	30 mins	25 mins	mp	v						
20	30 "	25 "	mp	v						
21	30 "	25 "	m	v						
22	40 "	30 "	mp	v						
23	35 "	30 "	m	v						
24	30 "	25 "	mp	v						
25	30 "	20 "	mp	v						
26	40 "	35 "	mp	v						
27	40 "	30 "	mp	v						
28	30 "	20 "	mp	sf						
29	30 "	25 "	mp	v						
30	30 "	25 "	mp	v						
31	30 "	25 "	mp	v						
32	50 "	45 "	p	vv						
33	15 "	10 "	m	f						
34	30 "	25 "	mp	v						
35	45 "	40 "	mp	v						
36	15 "	10 "	m	f						
37	15 "	10 "	m	sf						
38	40 "	35 "	p	vv						
39	35 "	30 "	mp	v						
40	20 "	15 "	m	f						
41	30 "	25 "	m	sf						
42	35 "	30 "	mp	v						

TABLE II

	TIME		Type		CULTURE			GRAM FILM		
	Water	Water and Beads			Direct	Water	Water and Beads	Direct	Water	Water And Beads
43	30 mins	25 mins	p	vv						
44	30 "	25 "	p	vv						
45	20 "	15 "	mp	v						
46	15 "	10 "	m	sf						
47	40 "	30 "	mp	v						
48	35 "	30 "	mp	v						
49	35 "	30 "	m	sf						
50	40 "	30 "	mp	v						
51	30 "	30 "	mp	v						
52	35 "	30 "	m	v						
53	30 "	25 "	mp	v						
54	20 "	10 "	m	sf						
55	30 "	25 "	m	v						
56	30 "	20 "	m	v						
57	30 "	20 "	m	v						
58	40 "	30 "	m	v						
59	45 "	35 "	m	v						
60	45 "	35 "	m	v						
61	35 "	30 "	p	sf						
62	35 "	30 "	p	sf						
63	40 "	30 "	mp	v						
64	35 "	30 "	mp	v						
65	45 "	40 "	mp	v						
66	45 "	40 "	m	sf						

TABLE II

	TIME		Type		CULTURE			GRAM FILM		
	Water	Water and beads			Direct	Water	Water and Beads	Direct	Water	Water and Beads
67	35 mins	30 mins	m	sf						
68	35 "	30 "	m	sf						
69	35 "	30 "	m	v						
70	35 "	30 "	m	v						
71	40 "	30 "	mp	v						
72	30 "	20 "	m	sf						
73	30 "	30 "	m	v						
74	40 "	35 "	m	v						
75	35 "	30 "	m	v						
76	35 "	30 "	m	v						
77	40 "	35 "	p	sf						
78	40 "	35 "	mp	v						
79	40 "	30 "	m	v						
80	40 "	30 "	m	v						
81	35 "	30 "	m	v						
82	45 "	35 "	m	v						
83	35 "	30 "	m	v						
84	40 "	35 "	mp	v						
85	45 "	35 "	m	v						
86	50 "	40 "	m	v						
87	25 "	15 "	m	sf						
88	40 "	30 m	v							
89	40 "	30 "	mp	v						
90	45 "	35 "	m	v						

TABLE II

	TIME		Type		CULTURE			GRAM FILM		
	Water	Water and Beads			Direct	Water	Water and Beads	Direct	Water	Water and Beads
91	30 mins	20 mins	m	sf						
92	30 "	40 "	mp	v	•	•	•	•	•	•
93	35 "	40 "	m	sf						
94	35 "	30 "	mp	v		•	•		•	•
95	40 "	35 "	m	v						
96	35 "	30 "	mp	v						
97	40 "	30 "	p	v	•	•	•	•	•	•
98	45 "	35 "	m	v		•	•	•	•	•
99	45 "	35 "	m	sf						
100	45 "	35 "	mp	v						

m - mucoid

mp - mucopurulent

p - purulent

v - viscid

vv - very viscid

f - fluid

sf - semi fluid

• - Pneumococci

• - Staphylococcus aureus or Staphylococci

• - Haemophilus influenzae

A blank space indicates that no pathogenic organisms were isolated from the specimen.

TABLE III

In this table is a record of the pathogenic organisms isolated on culture and present in the Gram film of random samples of untreated sputum, sputum homogenised by water and beads, and sputum homogenised by pancreatin, also the length of time taken by these reagents to produce homogenisation of the specimen.

TABLE III

TIME

CULTURE

	Water and Pancreatin Beads		TYPE		Untreated sputum						Homogenised with water and beads					Homogenised with pancreatin				
					1	2a	2b	2c	2d	2e	3a	3b	3c	3d	3e	4a	4b	4c	4d	4e
1	30mins	40mins	mp	sf																
2	30 "	55 "	mp	v																
3	35 "	60 "	mp	v																
4	20 "	25 "	p	sf																
5	15 "	25 "	m	f																
6	40 "	65 "	mp	v																
7	40 "	70 "	mp	sf																
8	60 "	125 "	mp	v																
9	45 "	65 "	mp	sf																
10	30 "	30 "	mp	sf																
11	25 "	60 "	m	v																
12	60 "	95 "	mp	vv																
13	30 "	60 "	mp	v																
14	45 "	95 "	m	v																
15	40 "	70 "	p	vv																
16	20 "	45 "	p	f																
17	45 "	70 "	m	v																
18	30 "	60 "	mp	v																
19	20 "	40 "	m	sf																
20	30 "	70 "	mp	v																
21	40 "	50 "	m	v																
22	30 "	50 "	p	v																
23	40 "	85 "	p	v																
24	35 "	70 "	p	v																
25	35 "	65 "	mp	v																
26	35 "	65 "	mp	v																
27	20 "	40 "	m	f																
28	40 "	65 "	mp	v																
29	45 "	70 "	mp	v																
30	40 "	60 "	mp	v																

TABLE III

CULTURES (CONT.)

	TIME		TYPE		Untreated sputum						Homogenised with water and beads					Homogenised with pancreatin				
	Water and Pancreatin Beads				1	2a	2b	2c	2d	2e	3a	3b	3c	3d	3e	4a	4b	4c	4d	4e
31	35 mins	55 mins	mp	v		•					•	•	•	•	•	•	•	•	•	•
32	30 "	50 "	mp	v	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
33	30 "	70 "	mp	v	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•
34	30 "	65 "	mp	v	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
35	30 "	50 "	mp	v	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
36	35 "	65 "	mp	v																
37	20 "	65 "	mp	sf		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
38	30 "	60 "	mp	sf	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
39	30 "	60 "	mp	sf	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
40	20 "	50 "	m	sf																
41	10 "	15 "	p	f	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
42	20 "	30 "	m	v	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
43	20 "	50 "	m	v																
44	40 "	65 "	p	v	•		•	•	•	•	•	•	•	•	•	•	•	•	•	•
45	45 "	75 "	p	v	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
46	35 "	70 "	mp	v	•	•	•		•	•	•	•	•	•	•	•	•	•	•	•
47	35 "	70 "	mp	v	•	•		•	•	•	•	•	•	•	•	•	•	•	•	•
48	35 "	75 "	mp	v	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
49	15 "	40 "	m	sf																
50	45 "	70 "	mp	v	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

mp. - mucopurulent
m. - mucoid.
p. - purulent

v. - viscid
vv. - very viscid
sf. - semi fluid
f. - fluid

• Pneumococci
• Staphylococcus aureus and staphylococci
• Haemophilus influenzae

1 = specimen taken direct from universal container; 2a to 2e = five random samples of untreated sputum
3a to 3e = five random samples of sputum homogenised by water and beads.
4a to 4e = five random samples of sputum homogenised by pancreatin.

TABLE III

FILMS

	TIME Water and Pancreatin Beads		TYPE	Untreated sputum						Homogenised with water & beads.					Homogenised with pancreatin.				
				1	2a	2b	2c	2d	2e	3a	3b	3c	3d	3e	4a	4b	4c	4d	4e
1	30 mins	40 mins	mp sf																
2	30 "	55 "	mp v																
3	35 "	60 "	mp v																
4	20 "	25 "	p sf																
5	15 "	25 "	m f																
6	40 "	70 "	mp sf																
8	60 "	125 "	mp v																
9	45 "	65 "	mp sf																
10	30 "	30 "	mp sf																
11	25 "	60 "	m v																
12	60 "	95 "	mp vv																
13	30 "	60 "	mp v																
14	45 "	95 "	m v																
15	40 "	70 "	p vv																
16	20 "	45 "	p f																
17	45 "	70 "	m v																
18	30 "	60 "	mp v																
19	20 "	40 "	m sf																
20	30 "	70 "	mp v																
21	40 "	50 "	m v																
22	30 "	50 "	p v																
23	40 "	85 "	p v																
24	35 "	70 "	p v																
25	25 "	65 "	mp v																
26	35 "	40 "	m f																
27	20 "	40 "	m f																
28	40 "	65 "	mp v																
29	45 "	70 "	mp v																
30	40 "	60 "	mp v																

TABLE III
FILMS(CONT.)

	TIME Water and Pancreatin Beads	Beads	TYPE		Untreated sputum						Homogenised with water and beads					Homogenised with pancreatin.				
					1	2a	2b	2c	2d	2e	3a	3b	3c	3d	3e	4a	4b	4c	4d	4e
31	35mins	55mins	mp	v																
32	30 "	50 "	mp	v																
33	30 "	70 "	mp	v																
34	30 "	65 "	mp	v																
35	30 "	50 "	mp	v																
36	35 "	65 "	mp	v																
37	20 "	65 "	mp	sf																
38	30 "	60 "	mp	sf																
39	30 "	60 "	mp	sf																
40	20 "	50 "	m	sf																
41	10 "	15 "	p	f																
42	20 "	30 "	m	v																
43	20 "	50 "	m	v																
44	40 "	65 "	p	v																
45	45 "	75 "	p	v																
46	35 "	70 "	mp	v																
47	35 "	70 "	mp	v																
48	35 "	75 "	mp	v																
49	15 "	40 "	m	sf																
50	45 "	70 "	mp	v																

mp. - mucopurulent
m. - mucoid
p. - purulent

v. - viscid
vv. - very viscid
sf. - semi fluid
f. - fluid

● Pneumococci
● Staphylococcus aureus and staphylococci
● Haemophilus influenzae

1 = specimen taken direct from universal container; 2a to 2e = five random samples of untreated sputum; 3a to 3e = five random samples of sputum homogenised by water and beads. 4a to 4e = five random samples of sputum homogenised by pancreatin.

Table IV

In this table is a record of the rate and degree of disintegration of deoxyribonucleoprotein fibrils in sputum homogenised by water, and water and beads. The degree of purulence of the sputum is also recorded.

A large number of pus cells = 3 points
A moderate number of pus cells = 2 points
A few pus cells = 1 point
An occasional pus cell = 0.5 point
Intact fibrils = 3 points
Moderate degree of disintegration = 2 points
Almost complete disintegration = 1 point
Complete disintegration = 0.5 point

TABLE 1V

	Time required for homogenisation		PUS CELLS			FIBRILS		
	Water	Water and Beads	Untreated	Water	Water &Beads	Untreated	Water	Water & Beads
1	30mins	25mins	.5	0	0	1	.5	.5
2	45 "	35 "	1	1	.5	1	.5	.5
3	45 "	35 "	1	.5	.5	1	.5	.5
4	50 "	40 "	2	2	1	2	1	1
5	30 "	25 "	1	.5	.5	.5	.5	.5
6	50 "	40 "	3	.5	.5	1	.5	.5
7	50 "	40 "	2	1	.5	2	.5	.5
8	50 "	40 "	2	.5	.5	1	.5	.5
9	40 "	30 "	.5	.5	.5	1	.5	.5
10	35 "	30 "	1	.5	.5	.5	.5	.5
11	55 "	45 "	2	1	1	3	1	1
12	35 "	30 "	1	.5	.5	.5	.5	.5
13	40 "	30 "	1	1	1	1	.5	.5
14	40 "	30 "	3	1	.5	2	1	.5
15	55 "	45 "	3	2	.5	3	1	1
16	45 "	40 "	1	.5	.5	2	.5	.5
17	45 "	35 "	1	.5	.5	1	.5	.5
18	55 "	45 "	3	1	1	2	.5	.5
19	45 "	35 "	2	1	.5	3	.5	.5
20	40 "	30 "	1	.5	.5	2	.5	.5
21	55 "	45 "	3	2	1	3	2	1
22	45 "	35 "	1	.5	.5	.5	.5	.5
23	35 "	30 "	1	.5	.5	.5	.5	.5
24	35 "	30 "	1	.5	.5	1	.5	.5
25	35 "	30 "	2	.5	.5	1	.5	.5
26	35 "	30 "	1	.5	.5	1	.5	.5
27	55 "	45 "	2	1	1	3	.5	.5

TABLE 1V

	Time required for homogenisation		PUS CELLS			FIBRILS		
	Water	Water and Beads	Untreated	Water	Water & Beads	Untreated	Water	Water&Beads
28	40mins	30mins	1	.5	.5	3	.5	.5
29	35 "	30 "	.5	.5	.5	.5	.5	.5
30	35 "	30 "	1	.5	.5	1	.5	.5
31	40 "	30 "	2	1	.5	2	.5	.5
32	45 "	35 "	2	.5	.5	3	.5	.5
33	35 "	30 "	1	.5	.5	1	1	1
34	35 "	30 "	1	.5	.5	1	.5	.5
35	45 "	35 "	2	.5	.5	2	.5	.5
36	45 "	35 "	2	.5	.5	2	.5	.5
37	30 "	30 "	1	.5	.5	1	.5	.5
38	30 "	30 "	1	.5	.5	1	.5	.5
39	30 "	30 "	1	.5	.5	1	.5	.5
40	45 "	35 "	1	.5	.5	2	1	.5
41	35 "	30 "	1	.5	.5	1	.5	.5
42	35 "	30 "	1	.5	.5	1	.5	.5
43	50 "	40 "	2	1	1	3	1	.5
44	35 "	30 "	1	.5	.5	1	1	.5
45	35 "	30 "	1	.5	.5	1	.5	.5
46	35 "	30 "	1	.5	.5	1	.5	.5
47	35 "	30 "	1	.5	.5	1	.5	.5
48	35 "	30 "	1	.5	.5	1	.5	.5
49	35 "	30 "	2	.5	.5	1	.5	.5
50	35 "	30 "	1	.5	.5	1	1	.5
51	35 "	30 "	1	.5	.5	1	.5	.5
52	55 "	45 "	3	1	.5	3	.5	1
53	40 "	30 "	2	1	.5	2	.5	1
54	35 "	30 "	1	.5	.5	1	.5	.5
			79.5	37.0	30.5	80	32	30.5

Table Va, b, c and d.

The size of zone of inhibition produced by disks impregnated with bile, sodium desoxycholate, sodium taurocholate, and optochin on 105 strains of pneumococci and 103 strains of Str. viridans using four methods of inoculation.

Table Va.

Inoculum - Loop of culture from blood organ plate

Reagent	Organisms	No zone o	Zone of 1-3mm	Zone of 4mm and upwards
Bile	Pneumo cocci	4	37	64
	Str. viridans	64	34	5
Sodium tauro cholate	Pneumo cocci	10	42	53
	Str. viridans	72	28	3
Sodium desoxy cholate	Pneumo cocci	4	19	82
	Str. viridans	65	29	9
Optochin	Pneumo cocci	7	7	91
	Str. viridans	78	21	4

Table Vb.

Inoculum Broth undiluted

Reagent	Organisms	No zone o	Zone of 1-3mm.	Zone of 4mm and upwards
Bile	Pneumo cocci	8	41	56
	Str. viridans	101	2	0
Sodium tauro cholate	Pneumo cocci	16	44	45
	Str. viridans	102	1	0
Sodium desoxy cholate	Pneumo cocci	10	29	66
	Str. viridans	100	3	0
Optochin	Pneumo cocci	9	19	77
	Str. viridans	103	0	0

Table Vc.

Inoculum Broth diluted 1:2

Reagent	Organisms	No zone o	Zone of 1-3mm.	Zone of 4mm and upwards
Bile	Pneumo cocci	2	32	71
	Str. viridans	102	1	0
Sodium	Pneumo cocci	8	25	72
tauro cholate	Str. viridans	103	0	0
Sodium	Pneumo cocci	3	13	89
desoxy cholate	Str. viridans	102	1	0
Optochin	Pneumo cocci	3	8	94
	Str. Viridans	103	0	0

Table Vd.

Inoculum Broth diluted 1:10

Reagent	Organisms	No zone o	Zone of 1-3mm	Zone of 4mm and upwards
Bile	Pneumo cocci	1	13	91
	Str. viridans	101	2	0
Sodium tauro cholate	Pneumo cocci	8	14	83
	Str. viridans	102	1	0
Sodium desoxy cholate	Pneumo cocci	1	5	99
	Str. viridans	101	2	0
Optochin	Pneumo cocci	2	5	98
	Str. viridans	103	0	0

PART 2

CHRONIC BRONCHITIS TRIAL OF THE MEDICAL RESEARCH COUNCIL

This is a trial of the effect of antibiotic treatment on specially selected patients suffering from chronic bronchitis.

The objects of the trial.

(i) To see whether a continuous antibacterial regime is effective in preventing exacerbations of bronchial infection in chronic bronchitis.

(ii) If it is effective, to see whether such prevention of exacerbations delays or prevents the development of respiratory disability.

(iii) To see whether the frequency and severity of exacerbations affects the rate of development of respiratory disability.

(iv) To compare the effects on these factors of two therapeutic regimes during exacerbations.

For acceptance in the trial the following criteria are required:-

1. The patient must be male between the age of 40 and 59, in regular employment. This group is chosen as it is possible for the Ministry of Pensions and National Insurance certificates to be studied if necessary.

The patient's permission is asked for this to be done.

2. He must be intelligent, willing to co-operate and likely to live in the area for three years.

3. He must have a history of bringing up phlegm first thing in the morning and during the day or night for at least three months in each of the past two years.

4. He should have had at least two illnesses characterised by the production of increased sputum in the three years before the date of admission, causing him to be off work for a total of at least three weeks.

5. The indirect maximum breathing capacity must be above 50 l/min.

6. He must <u>not</u> have (a) Cardiac disability (b) Diastolic blood pressure of 110mm. Hg. or over (c) A history of previous treatment for tuberculosis (d) Overt bronchiectasis (e) Progressive massive fibrosis (but simple pneumoconiosis is acceptable) (f) Pure asthma (g) Other diseases likely to cause progressive or recurrent disablement (h) A condition whose present management includes long-term chemotherapy (i) A history of sensitivity to chloramphenicol, sulphonamides, or tetracyclines.

The prophylactic antibiotic given in this trial is oxytetracycline, and the period of treatment is from mid-September to the end of April. Exacerbations are treated with either chloramphenicol or sulphonamide. This is also decided by random selection so there are four groups of patients as the table below shows:-

	Treatment of Exacerbations	
	Chloramphenicol	Sulphonamide
Oxytetracycline	I	III
Prophylaxis Dummy	II	IV

The trial commenced in April, 1959, and is still in progress. At regular intervals of from eight to twelve weeks, the patients are examined and their clinical condition assessed by the physician, and a specimen of sputum is investigated bacteriologically. The physician and the bacteriologist work independently of each other in that neither is cognisant of, or influenced by the findings of the other. Half the patients, chosen by random selection, are receiving an antibiotic. This is another factor which is unknown by the physician and the bacteriologist.

The present study covers the period from April 1959 to February, 1962.

MATERIALS AND METHODS OF BACTERIOLOGICAL INVESTIGATION

The protocol of the Medical Research Council investigation requires that the patients be seen at quarterly intervals. In addition to this three monthly examination, the patients in Edinburgh are also seen at eight weekly intervals, the two visits sometimes coinciding.

Clinical examination is undertaken on Friday evening. The specimen which is examined bacteriologically is the sputum expectorated during the first hour after rising on Monday morning. The patient is instructed to expectorate into a universal container. There is a variation between the method of despatch of the quarterly specimen and the eight weekly specimen. By close co-operation between the health visitors and the medical staff the quarterly specimens are received at the laboratory by mid-day of the Monday. In the case of the eight weekly specimens the patients send these by post, and thus arrival is delayed for twenty four hours. Whatever the method of delivery the specimens are treated immediately on arrival at the laboratory.

The first examination is a measurement of the amount and an assessment of the type of sputum.

The volume is measured as the height of the sputum in the universal container to the lower margin of the supernatant froth, the result being given in centimetres and millimetres. Three types of sputum are recognised - mucoid, muco-purulent and purulent.

A loopful of the sputum from the most purulent part of the specimen is inoculated on blood agar and heated blood agar, and Gram and Leishman films are made. The sputum is then rinsed in saline and an equal amount of sterile distilled water and about six glass beads are added. If the sputum more than half fills the bottle some of it is discarded. The specimen is then placed on the shaker and agitated for a minimum of thirty minutes. After homogenisation, blood agar and heated blood plates are inoculated and Gram and Leishman films made.

At the start of the trial the blood plates which had been inoculated with the homogenised

specimen of sputum were incubated under the influence of 10% carbon dioxide. This arrangement was amended when it was realised that it was not possible to assess whether a superior isolation rate of pneumococci was due to homogenisation, or the influence of carbon dioxide.

Two blood agar plates inoculated with the homogenised specimen are thus examined, one after incubation in the normal atmosphere and the other after incubation in an atmosphere containing 10% carbon dioxide.

The carbon dioxide is produced by the action of hydrochloric acid on chalk and the atmosphere in the container is humidified by placing a jar of water beside the plates.

The tests performed to identify pneumococci are bile solubility, using 10% sodium desoxycholate, optochin sensitivity using a disk impregnated with a 1:4000 solution,and inulin fermentation using a 1% solution of the sugar in Hiss's serum water with Andrade's indicator.

Mouse inoculation is not practised routinely but only in selected cases. In the later stages

of the trial typing sera from the State Serum Institute, Copenhagen, became available.

Haemophilus influenzae is identified on the morphology and colonial appearance, and the reaction to Gram's stain. Typing sera are available for the identification of capsulated organisms.

Staphylococcus aureus is identified by the colonial and morphological appearance and by coagulase production.

The following additional examinations were carried out:-

At four of the quarterly examinations all the sputum specimens, a total of 104, were cultured for a second time twenty four hours after arrival at the laboratory to see if there was any alteration in the findings.

On two occasions, June 1960 and December, 1960, the 52 specimens received were cultured on Sabouraud agar to favour the growth of fungi.

Sensitivity Testing

The sensitivities of the pathogenic organisms are tested to chloramphenicol and tetracycline. It was decided to use the disk

diffusion method with the provision that if results are obtained by this method which give rise to doubt, the sensitivity tests will be repeated by the plate dilution method in which doubling concentrations of the antibiotic are incorporated in the medium.

All organisms isolated from the sputum are recorded whether considered to be pathogenic or not. The amount of growth on culture is given as ± a few colonies, + a scanty growth, ++ a moderate growth, +++ a profuse growth. The number of organisms in the Gram film is recorded in a similar manner and the number of pus cells noted. The type of cell is identified from the Leishman film.

Nasal and axillary swabs are taken from each patient at his visit to the clinic. These are cultured on blood agar for the isolation of Staphylococcus aureus.

The strains of Staphylococcus aureus isolated are not phage typed as facilities for this examination are not available.

Estimation of Haemophilus influenzae antibodies in patients' serum.

Two rough strains of Haemophilus influenzae isolated in the laboratory were used. The organism was suspended in 0.1% formol saline and standardised to No.1 Brown's opacity tube.

The serum was diluted in doubling dilutions from 1:16 to 1:512. The tubes were placed in a water bath at 37°C. and readings were made after eighteen hours.

RESULTS

A total of thirty one patients were accepted for investigation in the trial. For reasons which are unknown, four of these were studied for a short period only at the start, leaving twenty seven patients who up to the present time are being investigated.

Bacteriological examination of the sputum of these twenty seven patients show that they fall into two main groups. Pathogenic organisms have been isolated from the sputum of seventeen from the start of the trial (April 1959) up to the present time (February 1962). In the remaining ten patients pathogenic organisms were isolated at the start of the trial but have since disappeared.

The following tables show an analysis of the organisms isolated from the twenty seven patients:-

Details of organisms isolated from the twenty seven patients.

Pneumococci isolated from	8 patients
Pneumococci and *Haemophilus influenzae* from	9 "

Haemophilus influenzae from	2	patients
Pneumococci and Staphylococcus aureus from	2	"
Pneumococci and Staphylococcus aureus and Haemophilus influenzae from	3	"
Proteus from	3	"
	27	

Organisms isolated from the seventeen patients from whom pathogens have been obtained since the start of the trial.

Pneumococci isolated from	4	patients
+ Pneumococci and Haemophilus influenzae isolated from	6	"
Pneumococci and Haemophilus influenzae and Staphylococcus aureus from	3	"
Pneumococci and Staphylococcus aureus from	1	"
Proteus from	3	"
	17	

Organisms isolated from the ten patients from whom at present no pathogens are being isolated

Pneumococci isolated from	4	patients
Pneumococci and Staphylococcus aureus from	1	"
Pneumococci and Haemophilus influenzae from	3	"
Haemophilus influenzae from	2	"
	10	

(+ In three of these pneumococci were isolated initially but have since disappeared)

Details of organisms isolated from four patients who did not complete the trial.

Pneumococci	1	patient
Pneumococci and *Haemophilus influenzae*	1	"
Haemophilus influenzae	1	"
Klebsiella pneumoniae	1	"
	4	

Types of Pneumococci isolated.

Type 2	1	patient
Type 3	2	"
Type 6	2	"
Type 9	1	"
Type 17	1	"
Type 32	4	"
Type 34	2	"
	13	

Types of pneumococci which continue to be isolated from patients.

Type 2	1	"
Type 3	2	"
Type 6	1	"
Type 9	1	"
Type 17	1	"
Type 32	3	"
Type 34	2	"
	11	

Not all the pneumococci which were isolated have been typed as typing sera were not available at the start of the investigation. The type of pneumococcus remained the same in all patients with the exception of one where there was a change from type 6 to type 32. Only one type was isolated from each patient in a single examination.

The strains of Haemophilus influenzae isolated from all patients were without capsules and so could not be typed.

Variations in isolation rate during the year.

It was noticed that pathogenic organisms were not isolated at a constant rate throughout the year. In the 17 patients from whom pathogens are still isolated, the winter isolation rate for pneumococci and Haemophilus influenzae is 7.5 times greater than the summer rate. In the 10 patients from whom at present (February 1962) no pathogens are isolated the winter isolation rate was 4 times that of the summer isolation rate. There is no apparent variation

in the incidence of Staphylococcus aureus during the summer or winter.

The division between winter and summer has been taken as the periods with and without antibiotic therapy. That is, winter is from mid-September to the end of April and summer is the remainder of the year.

The volume and type of sputum in relation to the pathogenic organisms isolated

It should be remembered in this connection that the volume of sputum recorded is that expectorated for the first hour after rising and no information is available as to the amount produced during the rest of the day and night.

In 25 of the 27 patients the volume of sputum has remained very constant throughout the trial and the variations in quantity are slight. In these patients there was no apparent relationship between the isolation of pathogens and the amount of sputum produced.

Two of the patients produced varying amounts of sputum and here an increased volume was associated with the isolation of pneumococci.

Relationship of purulence of the sputum to the isolation of pathogens.

In assessing the degree of purulence of the sputum both the macroscopic and microscopic appearance of the sputum was studied. It was considered that the microscopic appearance gave a more detailed picture of the amount of pus cells present. Smears of the sputum were stained with Leishman in order to identify the type of pus cell present. In only three patients were there three single instances of unusually large numbers of eosinophil polymorphs. Otherwise the predominant cells present in the films were neutrophil polymorphs.

The presence of pathogenic organisms was associated with the production of mucopurulent or purulent sputum.

The following table indicates the relation of purulence in the sputum to the isolation of pneumococci and Haemophilus influenzae.

	Mucopurulent	Purulent
Pneumococci	78%	22%
Haemophilus influenzae	40%	60%
Pneumococci and Haemophilus influenzae	33%	66%

Staphylococcus aureus appeared to be evenly distributed between mucopurulent and purulent sputum. There were no isolations of pneumococci, Haemophilus influenzae or Staphylococcus aureus from mucoid sputum.

There are three patients in the trial from whom Proteus has been isolated from each specimen examined in the trial. The character of the sputum in all cases is very similar. It is fluid in consistency, greyish yellow in colour, giving the impression of macroscopic pus. Microscopic examination however reveals that there are very few pus cells present. Two of the patients have produced large volumes of sputum throughout. The volume of sputum in the third is smaller in amount but like the others has remained very constant.

Study of the isolation rate of pathogenic organisms from homogenised sputum and untreated sputum

Pneumococci.

Pneumococci were isolated from the sputum on 85 occasions from the homogenised specimen, and on 67 occasions from the untreated specimen.

Pneumococci were not isolated from the untreated specimen if they were not recovered from the homogenised sputum.

Haemophilus influenzae

There are two factors to be considered in assessing the isolation rate of Haemophilus influenzae. The first is homogenisation of the sputum and the second the advantage of heated blood agar over blood agar as a culture medium.

Isolations from homogenised sputum

From heated blood agar on 47 occasions

From blood agar on 30 occasions

Isolations from untreated sputum

From heated blood agar on 40 occasions

From blood agar on 27 occasions

The influence of carbon dioxide on the growth of pneumococci.

The beneficial effect of carbon dioxide on the growth of all pneumococci has been noticeable throughout. The method used for the production of the carbon dioxide is the action of hydrochloric acid on chalk. In this method a certain amount of moisture appears in the container and it is observed that this improves the growth of the

organism. The heaviness of the growth appears to be in direct proportion to the degree of condensation. To further this a jar of water is placed in the container.

During the second year of the investigation it was observed that pneumococci isolated from three patients grew only on culture with the addition of 10% carbon dioxide.

There was on the plate incubated under these conditions a profuse growth of the organism, in marked contrast to a complete lack of growth on the plate incubated under normal aerobic conditions.

The first of these organisms was a type 2 pneumococcus. From the beginning this pneumococcus had been isolated without difficulty from several specimens until January, 1961, when it was found to grow on culture only when carbon dioxide had been added. Since that time these conditions have been essential for growth. The sputum from this patient was inoculated in a mouse when pneumococci were cultured from the heart blood. These organisms grew only in the presence of carbon dioxide.

The second of the pneumococci growing under these circumstances is a type 17 organism. It was cultured regularly without difficulty from the sputum until October, 1960, when it was found that the addition of carbon dioxide was essential for growth. It has been cultured frequently since then under the same conditions. The organism isolated by mouse inoculation required the same conditions of growth.

The third pneumococcus in this group is a type 3 which grew well under ordinary conditions until December, 1960, when the addition of carbon dioxide became essential for growth. These conditions have continued to be necessary both with the organism isolated from the sputum and those isolated by mouse inoculation.

In all the specimens under examination pneumococci were present in large numbers in the Gram film of the sputum.

Relation of the age of the sputum specimen to the Pathogenic organisms isolated.

At four of the quarterly examinations all the sputum specimens, a total of 104, were cultured for a second time twenty four hours after arrival at the laboratory.

All pathogenic organisms isolated on the first day were also isolated on the second day.

In four of the cases already described where carbon dioxide was required for the growth of the organism, the sputum when inoculated in a mouse was three days old but typical pneumococci were cultured from the heart blood of the animal.

Incidence of isolation of Staphylococcus aureus

The incidence of isolation of Staphylococcus aureus from the sputum is not high. Five patients have produced growths during the course of the trial. In three of these this was on one occasion only. One patient produced a culture on four occasions and one patient on three occasions. These isolations occurred at irregulare intervals in the course of the investigation. Staphylococcus aureus was isolated from the nasal swabs of five patients. In four patients this was a single isolation and in the remaining patient there were two positive cultures at an interval of 18 months. Only one patient yielded Staphylococcus aureus from both the sputum and nasal swab and

these were at separate times. Staphylococcus aureus was isolated from the axillary swab of one patient. This was the only isolation of the organism from this patient.

Results of investigation for the presence of Haemophilus influenzae antibodies in the patients' serum

The serum of twenty four patients was examined for Haemophilus influenzae antibodies on a single occasion in January, 1962. The following are the results obtained:-

No agglutination obtained with the patients' serum - 7 patients

Details of bacteriological examinations of these patients:-

B. proteus isolated throughout - 2 patients

Pneumococci isolated at start of trial only - 2 "

Pneumococci and Haemophilus influenzae at start of trial only - 1 patient

Pneumococci isolated throughout trial - 2 patients

7

Patient's serum agglutinating Haemophilus influenzae in dilution 1:32 - 1 patient

Details of bacteriology -

B. proteus isolated throughout trial — 1 patient

Patient's serum agglutinating *Haemophilus influenzae* in dilution 1:64 — 4 patients

Details of bacteriology -

Pneumococci isolated at start of trial — 1 patient

Pneumococci and *Haemophilus influenzae* isolated at start of trial — 1 "

Pneumococci isolated throughout trial — 1 "

Pneumococci and *Haemophilus influenzae* isolated throughout trial — 1 "

4

Patient's serum agglutinating *Haemophilus influenzae* in dilution 1:128 — 5 patients

Details of bacteriology -

Pneumococci at start of trial — 1 patient

Pneumococci and *Haemophilus influenzae* at start of trial — 1 "

Pneumococci isolated throughout trial — 1 "

Pneumococci and *Haemophilus influenzae* isolated throughout trial — 2 patients

5

Patient's serum agglutinating Haemophilus influenzae in dilution 1:256 - 3 patients

Details of bacteriology -

Pneumococci at start of trial - 1 patient

Pneumococci and Haemophilus influenzae throughout trial - 2 patients

3

Patient's serum agglutinating Haemophilus influenzae in dilution 1:512 - 3 patients

Details of bacteriology -

Pneumococci and Haemophilus influenzae at start of trial - 1 patient

(There was only one single isolation of Haemophilus influenzae)

Pneumococci isolated at start of trial - 2 patients

3

Patient's serum agglutinating Haemophilus influenzae in dilution greater than 1:512 - 1 patient

Details of bacteriology -

Pneumococci and Haemophilus influenzae isolated at start of trial - 1 patient

1

The control serum in this investigation agglutinated Haemophilus influenzae in dilution 1:32.

Results of Sensitivities of pathogenic organisms to tetracycline and chloramphenicol.

Throughout the trial the sensitivities of the organism were tested by the disk diffusion method.

There were a total of 85 isolations of pneumococci and a total of 47 isolations of Haemophilus influenzae. All these organisms were sensitive to the action of tetracycline and chloramphenicol.

All strains of Staphylococcus aureus - a total of 17, were sensitive to chloramphenicol. Five strains of Staphylococcus aureus isolated from the sputum were resistant to tetracycline. All other strains of Staphylococcus aureus isolated from the sputum were sensitive to the drug. Of the five tetracycline resistant strains, three were isolated from one patient, and two from one patient. These were the only isolations of Staphylococcus aureus from these patients.

All strains of Staphylococcus aureus isolated from nasal and axillary swabs were sensitive to chloramphenicol and tetracycline.

Results of findings in Gram film.

Pathogenic organisms present in Gram film.

It was found that when pathogenic organisms were isolated on culture they were also present in the film.

Other organisms isolated from the sputum.

Throughout the trial the almost complete absence of coliform organisms was noticeable. This observation does not include the three patients from whom Proteus has been isolated throughout.

In four patients there was a single isolation of Bact. coli and this was a scanty growth of the organism.

One patient yielded a growth of Proteus on two different occasions.

Monilia has also been noticeably absent. Four patients produced scanty growths on single occasions during the trial. These were isolated on blood agar.

In June, 1960, and December, 1960, 52 specimens received at the quarterly trial were inoculated on Sabouraud agar. From the sputum

of nine of the 26 patients growths of Monilia were obtained at one or both examinations. The results are in the following table:-

	June	December
Patient 1	+	+++
" 2	-	+
" 3	-	+
" 4	+	+
" 5	-	+
" 6	-	+
" 7	+	+++
" 8	++	++
" 9	+	"

+ = a scanty growth: ++ = a moderate growth:
+++ = a profuse growth.

In all except one patient where Monilia stallatoidea was found, the fungus isolated was Monilia albicans.

The other commonly isolated organisms are Strept. viridans and N. catarrhalis. They have appeared throughout the trial in varying numbers.

Seven patients produced profuse growths of Stept. viridans associated with purulent sputum. The Gram film of these sputa showed large numbers of streptococci. No pathogenic organisms were isolated at these examinations.

N. catarrhalis was found commonly on culture. It was observed that this organism although frequently scanty in the film was profuse in culture.

On three occasions in different patients a profuse growth of N.catarrhalis was associated with purulent sputum. On these examinations the Gram film showed large numbers of pus cells with numerous intra and extra cellular neisseriae. No pathogenic organisms were isolated at these examinations.

The identification of pneumococci by bile solubility, optochin sensitivity and inulin fermentation.

There were three strains of pneumococci which had the typical morphology and colonial appearance of the organism, but which were insensitive to optochin. The sputum was inoculated into a mouse when pneumococci with similar

characters were isolated from the heart blood.

Mouse inoculation

Mouse inoculation was not done as a routine but it was carried out in the above examples and in six cases where pneumococci appeared to be present in the film of the sputum but did not grow on culture. Inoculation of the sputum in these cases produced a growth of the organism from the heart blood of the mouse.

Relation of fog to the isolation of pathogenic organisms.

From information received from the Meteriological Office, it was known there was severe fog on 19th November, and 24th, 27th and 28th December, 1960, and in 1961 on November, 10th, 18th, 19th and 22nd; also on December, 19th, 20th and 21st.

In 1960 12 out of 27 patients produced pathogens in the sputum on the first visit after the foggy period which had been approximately one week to ten days previously. There were 7 isolations of pneumococci and 5 of Haemophilus influenzae. On the previous examination no pathogens were isolated from the sputum. In 1961

12 out of 26 patients produced pathogens from the sputum in the visit after the foggy period which was 7 to 10 days before. There were 3 isolations of Haemophilus influenzae, 5 isolations of pneumococci, 1 isolation of Haemophilus influenzae and pneumococci, 1 isolation of Haemophilus influenzae and Klebsiella pneumoniae, and 2 isolations of Staphylococcus aureus.

From only one of these twelve patients had a pathogenic organism (Haemophilus influenzae) been isolated at the previous examination.

DISCUSSION

There are many factors in this trial which are as yet unknown, and although some of the conclusions drawn from the bacteriological results must of necessity be surmise, some definite facts do arise.

There has been a high isolation rate of potentially pathogenic organisms. Considering all the patients who were accepted for the study, out of a total of 33 there were 30 from whom pneumococci and Haemophilus influenzae were cultured. There have been several reports of the isolation of these organisms from the sputum

of patients with chronic bronchitis (May 1953a, 1953b, 1954; May and Oswald, 1956; Helm et al, 1954; Stuart Harris, et al., 1953; Mulder et al., 1952; Elmes et al., 1953; Edwards et al., 1957; Murdoch et al., 1959; Brumfitt and Willoughby, 1958; Buchanan et al., 1958).

The significance of Haemophilus influenzae has been stressed by Mulder and colleagues (1952) who reported that this organism could be isolated from 84% of patients with chronic mucopurulent bronchitis, which was nearly always associated with bronchiectasis. The studies of May (1954) where Haemophilus influenzae was present in 80-90% of cases of purulent bronchitis confirm this finding. In his earlier works (1953 a & b) equal importance was given to pneumococci and Haemophilus influenzae but in the later study Haemophilus influenzae was isolated four times as frequently as pneumococci. This high isolation rate of Haemophilus influenzae was again found in a later investigation (May & Oswald 1956). In a study of 17 patients with chronic bronchitis, Helm and his colleagues (1954) isolated Haemophilus influenzae from 94% of cases. 81% of the cases studied by Buchanan and colleagues (1958) were infected with Haemophilus influenzae.

In the present study pneumococci were isolated from 22 and Haemophilus influenzae from 16 of the 33 patients indicating that the pneumococcus was the more frequent infecting agent.

An improved bacteriological technique was a reason put forward by May for the increased numbers of Haemophilus influenzae isolated. The patients studied by Mulder and colleagues, (1952), Helm and others (1954) and May and Oswald, (1956), were described as advanced chronic bronchitics, and although the adjective "advanced" was not applied by May in 1954 the patients then did have some degree of dyspnoea. The significance of Haemophilus influenzae in bronchiectasis was pointed out by Mulder (1940). In a study of the incidence and significance of Haemophilus influenzae in chronic bronchiectasis, Allison and his associates (1943) isolated Haemophilus influenzae in 63% of patients suffering from the disease.

In the present trial the patients selected for investigation have been in the earlier stages of chronic bronchitis with no overt bronchiectasis, and it may be that Haemophilus influenzae is

isolated more frequently when the condition has become advanced and inflammatory changes and over-activity of the secreting elements are present.

The incidence of Haemophilus influenzae is high in purulent sputum. May in his study in 1954 found a frequency of isolation of 80-90% of Haemophilus influenzae from purulent sputum. A correlation between purulent and mucoid sputum and the presence or absence of pathogens particularly Haemophilus influenzae was found by Helm and associates (1954). Edwards and others (1957) isolated Haemophilus influenzae from 60% to 70% of purulent sputum. More than half of all purulent sputum examined from chronic bronchitics yielded a growth of Haemophilus influenzae (Murdoch and others 1959).

This association between purulent sputum and the presence of Haemophilus influenzae was observed when antibiotic treatment directed at the organism was given. The change from purulent sputum to mucoid sputum by suitable chemo-therapy was observed by May (1953b) who found that relapse occurred rapidly after discontinuance of the therapy. The degree of purulence of the sputum

was considered by Murdoch and his colleagues (1959) as a useful guide to the efficiency of the antibiotic therapy.

The antibiotic treatment received by the patients in the trial is unknown but the relation between purulent sputum and the isolation of Haemophilus influenzae is in agreement with the findings discussed above. 60% of Haemophilus influenzae were isolated from purulent sputum and 40% isolated from mucopurulent sputum. At no time was there a growth of the organisms from mucoid sputum.

All the strains of Haemophilus influenzae which were isolated were unencapsulated. Mulder (1952) found that of the strains isolated from adults, 95% were unencapsulated and 5% capsulated. If, as would appear, unencapsulated Haemophilus influenzae is a pathogen of the bronchial mucous membrane, the source of the infection is of interest and importance. In health the bronchial tree is free of the presence of pathogenic organisms. By using a special technique of bronchial swabbing Brumfitt and his colleagues (1957) were able to demonstrate the sterility of the bronchial tree.

In contrast the naso-pharynx harbours a large number of organisms some of a potentially pathogenic nature among which are unencapsulated Haemophilus influenzae, pneumococci, Staph. pyogenes, Strept. pyogenes, and Friedlander's bacillus (Stuart Harris and colleagues 1953). These may be present in varying numbers at different seasons and conditions, in the general public. A carrier rate of Haemophilus influenzae of 40-80% in the naso-pharynx was found by Rosher (1939) who found no increased incidence of the organism in the naso-pharynx during a cold, but considered there wasasuggestion in certain people that it was more frequently present in the nose in the later stages of such an infection.

There is then an ample source of pathogenic organisms which may assume a pathogenic role in a bronchial mucosa of lowered resistance.

As has already been discussed there was a noticeably high isolation rate of pneumococci in the present study. A predominance of pneumococci has rarely been observed in bacteriological studies of the sputum of chronic bronchitics.

Stuart Harris and colleagues (1953) reported a high isolation rate of pneumococci. In this investigation pneumococci were isolated from 50% of cases and Haemophilus influenzae from 15%. This finding may have been influenced by the employment of mouse inoculation in some cases, and the failure to use a selective medium for the isolation of Haemophilus influenzae. In the opinion of Brumfitt and Willoughby (1958), the high isolation rate of pneumococci observed by Stuart Harris and colleagues (1953) was possibly due in part to some of the strains of pneumococci having originated from the throat. From a comparison of the bacteriology of bronchial swabs and sputum and throat swabs, Brumfitt and colleagues (1957) concluded that where a pathogenic organism was absent from the bronchial swab but present in the sputum and throat swab, it was a contaminant from the throat. In this study pneumococci were the organisms which most frequently appeared as contaminants.

In the present study as throat swabs were not taken, it is not possible to estimate the possible degree of contamination. However as isolation of pneumococci was associated with purulence of the sputum, indicating an inflammatory reaction, it is considered that they should be regarded as infecting agents. In addition it was possible to type certain of the strains isolated, which showed that the type isolated from the sputum remained constant, indicating that one type had gained access to the lower respiratory tract. If the pneumococcus was derived from the upper respiratory tract, isolation of more than one type might have been expected. Masters and colleagues (1958) showed a variation in the pneumococcal types isolated from carriers.

A consideration of the types of the strains isolated can give some indication of the potential danger caused by their presence. As with Haemophilus influenzae, the pneumococci isolated from the sputum of chronic bronchitic patients are considered to be derived from the patient's own naso-pharynx. This conclusion is based on a

comparison of types isolated from chronic bronchitic sputum and those found in the throat and nose of healthy people. Heffron (1939) gives the carrier rate in healthy people as type 1 0.4%, type 3 10.5% and the remaining types 34.9%.

Types commonly found in the respiratory tract of carriers are 6,19 and 23 (Cruickshank and colleagues 1960). Type 14 is also found commonly in carriers (Masters and his associates 1958).

The number of types identified in this trial is too small to analyse statistically but the overall impression is that they are following the generally accepted pattern.

The regular and frequent isolation of two type 3 pneumococci from two patients and a type 2 pneumococcus from one patient raises the question of the epidemiological significance of these organisms. As well as the recognised virulence of these types in respiratory infections, the invasive nature of type 2 makes the question more pertinent. In addition to the hazard to the

patient himself there is also the possibility of spread to others who are in close contact. A study of the family history and living conditions could be of some interest. In a study of the spread of respiratory disease among families, Brimblecombe and colleagues (1958) found that the transfer rate of pneumococci isolated from the nose and throat rose significantly with the degree of crowding. Where new types of pneumococci were introduced into the family it was found that the school children and the fathers, the members of the group who had the closest most frequent contacts outside, were the most frequent offenders. Masters and associates (1958) found that thirteen new types were introduced into nine families. A study of the family history of sickness and the living conditions of these patients could be of interest. Examination of the bacterial flora of the upper respiratory tract of the patient and his immediate family circle might yield information as to a possible source of the organism. There is a high nasal carrier rate of pneumococci in young children (Masters et al 1958).

The presence of two type 6 organisms is not surprising as this type is commonly found in healthy carriers. Prolonged carriage of this type is common (Masters 1958). Of the other types isolated, of interest is the presence of four strains of type 32. It has been observed in other studies in this hospital that type 32 has been isolated with some frequency from the sputum of patients with chronic bronchitis and it may be that this type is not unusual in this part of the country.

There are three patients from whom Proteus has been isolated throughout the trial. The regularity of the isolation of this organism has been a marked feature and has been associated with the same type of sputum in all patients. This has been of a mucoid, fluid character, profuse in amount in two, and somewhat less in quantity in the other. The volume has remained in all three patients very constant all through the investigation. Sensitivity tests have shown that the organisms are resistant to chloramphenicol, tetracycline and sulphonamide, so that

if these patients are receiving an antibiotic they are not likely to derive benefit from it. Without clinical information it is difficult to say what importance these organisms have. The absence on microscopic examination of a large number of pus cells would give the impression that there is not a gross inflammatory reaction present, yet it is felt that some consideration should be given to the presence of these organisms in large numbers on culture and in the Gram film. The question arises as to the source of these organisms. As they have been present since the first examination it does not appear that they are colonising the bronchial tract following elimination of other bacteria by chemotherapy. The possibility of a focus of infection perhaps in a sinus should not be overlooked. It is of interest that culture of the nasal swabs from these patients has not yielded growths of B. Proteus. The site of swabbing was the anterior nares which could account for a negative finding if the site of the infection was a sinus. Snuff has been studied as a source of infection in chronic bronchitis by Dygert (1957) who cultured Proteus from it.

The smoking habits of the patients areunknown but this source of infection although possible is not likely.

The presence of antibodies to Haemophilus influenzae in the serum of patients suffering from chronic bronchitis is another indication of the significance of the organism in the disease. This examination was undertaken on one sample of serum from 24 patients during the winter of 1961. No comparison is therefore available with the antibody level during the early part of the trial or in the summer months. In spite of these limitations some interesting facts have emerged, although there is such complexity in the antigenic structure of the Haemophilus influenzae that it is difficult to assess them completely.

The control serum throughout this study agglutinated Haemophilus influenzae in a dilution of 1:32. If this reading is taken as normal, the details of the eight patients whose serum agglutinated up to this dilution is of interest.

In only one case was Haemophilus influenzae isolated from the sputum and this was at the start of the trial. It is of interest that the serum of the three patients with mucoid sputum at all examinations gave either negative readings or a very low titre. When considering the higher antibody titres, interpretation of the results shows some apparent anomalies, if infection of the sputum with Haemophilus influenzae is taken as the necessary requirement of agglutination of the serum.

There were a total of fifteen patients in this group and of these there were nine from whom Haemophilus influenzae had been isolated from the sputum. From the remaining six only pneumococci had been isolated. An obvious explanation for this is a faulty bacteriological technique but it is not felt that this is the answer.

There is no standard technique for this procedure and it is thus not possible to compare results with those of other workers. Glynn in 1959 detected antibodies by titration against tanned red cells and obtained very high titres.

He found no relation between the antibody titre or severity of the disease, the duration and the presence of active infection. No reference was made in his work to the purulence of the sputum and the level of the antibody titre.

Other writers stress the significance of raised titres but do not give details of the method, and Murdoch and associates (1959) found the antibody titre did not rise above 1:240 in patients from whom Haemophilus influenzae had not been isolated. In a study of the use of Haemophilus influenzae vaccine, Brown and Wilson (1959) studied the effect of the vaccine on the level of the titres but do not give particulars of readings or technique.

Although the presence of a raised antibody titre is confirmatory evidence of the role of Haemophilus influenzae in chronic bronchitis, there are very wide gaps in our knowledge of the subject. It is not known if this is a manifestation of the early stage of the disease and remains even after therapy has removed the organism from the sputum or if it appears only after the condition has been established.

The invasive nature of the Haemophilus influenzae was recognised by Hers and Mulder (1953) who demonstrated the organism in the basement membrane while the bronchial epithelium was unimpaired. This incursion of the Haemophilus influenzae is considered by Murdoch and co-workers (1959) to be a contributing factor in the production of antibodies. Present knowledge of the antigenic structure of the organism is so limited that there are many grounds for error. It is however a subject of great interest and would provide a field for fruitful and rewarding study.

Infection of the sputum with Staphylococcus aureus has not fortunately been a problem in this investigation. In a trial of long term chemotherapy in chronic bronchitis May (1956) found a quarter of the patients were carriers of antibiotic resistant coagulase positive staphylococci at the end of the study. There have been only transient isolations of Staphylococcus aureus in this trial. As far as is known none of the patients have been hospitalised during the period which may be contributing factor to the paucity of isolations.

No evidence of increased resistance to chloramphenicol and tetracycline has been observed in the pneumococci and Haemophilus influenzae isolated. Sensitivity testing was performed by the disk diffusion method and as this appeared to give satisfactory results the plate dilution method was not employed. Five of the strains of Staphylococcus aureus isolated from the sputum were resistant to tetracycline but it was considered they were only temporary inhabitants of the respiratory tract.

The detrimental effect of air pollution and fog in the causation of chronic bronchitis has been indicated by the greater incidence of the disease in industrial areas and by the increased mortality following periods of severe fog. (Oswald, 1958; Logan, 1953b; Martin and Bradley 1960).

It is noticeable that there was a much higher isolation rate of Haemophilus influenzae and pneumococci during the winter months, than in the summer. A much higher carrier rate of

pneumococci was found during the winter months by Masters and his co-workers (1958). In the patients in this study from whom pathogens are still being isolated, the incidence is 7.5 times greater in the winter than in the summer. As the hazard of fog is a serious threat to the health of the chronic bronchitic, it was considered of interest to see if this was related to the isolation of pathogens. From information received from the Meteriological Office, it was known there was severe fog on 19th November, 1960, and 24th, 27th and 28th December, 1960, and in 1961 on November 10th, 18th, 19th and 22nd; also on December 19th, 20th and 21st. Sputum examined seven to ten days after the fog in 1960 yielded pathogenic organisms from 12 out of 27 patients. In the previous examination which was before the onset of fog no pathogens had been isolated from these patients. Culture of the sputum at the first examination after the fog in 1961 showed pathogens in 12 out of 26 patients examined. Only one of these twelve patients had pathogenic organisms in the sputum at the visit preceding

the fog. The particular danger of fog is mentioned by Elmes and colleagues (1955) who state that persons infected by Haemophilus influenzae are more susceptible to its effects. A higher isolation rate of Haemophilus influenzae was found by Murdoch and colleagues (1959) during the second year of the study when there was a higher incidence of foggy weather.

The inhalation of an irritant substance such as fog produces an excess of mucus with an associated increase of goblet cells. This increase of goblet cells entails a reduction in the ciliated cells with the result that the secretion is not removed by the ciliary streams and forms a focus for the proliferation of bacteria.

The results in the present trial would indicate that some of the patients have suffered adverse effects from the effect of the winter weather.

The volume and consistency of sputum produced by patients suffering from chronic bronchitis may vary according to the season and the activity of the disease. Oswald (1958) in a

study of a thousand specimens of sputum found there was an increase in volume during the winter and during exacerbations of the illness. This larger volume was more marked in exacerbations, 863 of the patients having a volume of more than 1 oz. at these times. The purulence of the sputum was also greater during exacerbations. More patients produced sputum during the winter but there was not a marked increase in the amount of pus. Fletcher and Tinker (1961) found that chest illnesses were more frequent with increasing sputum volume. These were early morning specimens.

As in this study only the early morning specimen is examined, it is not possible to make an assessment of this nature. It was noticeable that with the exception of two, the type and volume of this particular specimen did not show a variation throughout the trial, the amount produced at the start of the investigation being very similar to that produced at a later date. There was no association between the volume of

the sputum and the isolation of pneumococci. It would be necessary to have information of the total volume produced during the twenty four hours before a complete correlation could be made.

In a survey of this nature the patients under observation are generally out-patients, and so the problem of receiving a fresh specimen of sputum arises. There were two types of specimens in the present series, those which were produced on the day of examination and those received by post the following day. There did not appear to be a difference in the isolation rate of pathogens between the two groups and indeed some pneumococci survived a third day as evidenced by their isolation by mouse inoculation. To provide a direct comparison, specimens from four of the quarterly examinations were cultured on two consecutive days without any reduction in the rate of isolation of pathogens. This result would indicate that a

delay of a day would not be detrimental, and that delivery of the specimen by post is a satisfactory and suitable one for patients who are employed and unable to attend during working hours.

A comparison was made of the isolation rate of pathogenic organisms from untreated sputum, and sputum which had been homogenised by shaking with water and beads. The number of isolations of pneumococci and Haemophilus influenzae was greater from the homogenised sputum, and this is in agreement with May (1952) (1953a) who recommended treatment of the specimen. The method used by him was liquefaction with pancreatin.

For the isolation of Haemophilus influenzae heated blood agar was used as a selective medium and this was more effective in yielding a culture of the organism than ordinary blood agar. Although Haemophilus influenzae does grow on ordinary blood agar it is advisable to employ selective media. Stuart Harris and colleagues (1953) considered that Haemophilus influenzae was missed in some cases when only ordinary blood agar was used.

All pneumococci isolated were in addition to their morphology and colonial appearance identified by their reactions to sodium desoxycholate, inulin, and optochin. There were a few organisms which were insensitive to optochin.

These optochin resistant pneumococci when inoculated intraperitoneally into mice were all shown to be virulent organisms.

Mouse inoculation was not practised routinely but only in selected cases. The isolation rate of pneumococci might have been higher if all specimens had been inoculated into mice.

It is considered that the examination of the sputum of chronic bronchitics by cultural and staining methods gives a very good indication of the degree of infection present. It is of interest that four of the sputa inoculated into mice in addition to pneumococci yielded growths of Haemophilus influenzae from the heart blood. This may be an indication of the invasive nature of the organism. Stuart Harris and colleagues (1953) found Haemophilus influenzae often mouse virulent in cases of bronchiectasis.

When examining plates for the presence of Haemophilus influenzae, the characteristic odour of the organism was of assistance, particularly where there was a mixed growth of other flora. To define this odour is rather difficult as it is quite distinctive. It has a pungent acrid quality with a background somewhat reminiscent of yeasts.

The addition of carbon dioxide to the atmosphere was found to be very beneficial to the growth of all strains of pneumococci. The appearance in the later stage of the trial of three strains of pneumococci, for which these conditions became obligatory, is of interest. It emphasises the need of a careful examination of a Gram film without which these organisms would have been overlooked and also the question as to their relation to the poor pulmonary ventilation found in chronic bronchitics in the later stages of the disease.

A moist atmosphere was also found to enhance the growth of the pneumococcus. This was also the opinion of Masters and his colleagues (1958).

A careful examination of a Gram film was a good indication of the organismal content of the sputum. In addition to the identification of pathogenic organisms the amount of pus could be estimated. This was sometimes at variance with the macroscopic appearance, a finding most marked when Proteus was the infecting agent. The importance of a preliminary Gram stain is stressed by Mulder (1956). As there may be an asthmatic element in certain cases diagnosed as chronic bronchitis it is necessary to identify the pus cells which are present in the sputum (May 1954). Although in a few specimens there was an increase in the numbers of eosinophil polymorphs this was with a few patients at widely separated intervals, and it did not appear to be of significance.

The other organisms isolated from the sputum were Strept. viridans and neisseriae. They were isolated regularly both with and without the presence of pathogenic organisms. On a few occasions they were associated with the production of purulent sputum. There were three specimens of purulent sputum where neisseriae were very numerous in the Gram film and showed the intracellular distribution of an acute infection.

Generally culture of neisseriae was much more prolific than the numbers of organisms seen in the film would lead one to expect. There may be occasions when the resistance of the host is lowered for some reason allowing such organisms to produce a low grade infection.

There was a noticeable lack of growth of coliform organisms. This finding is unusual as some of the patients were receiving oxytetracycline. In a study of long term prophylactic administration of tetracycline to bronchitic patients, Buchanan and associates (1958) found that coliforms grew profusely particularly in the patients receiving the drug. The reason why in this series there was no overgrowth of Gram negative bacilli is not clear but it is a satisfactory finding as these organisms may, in conditions of reduced resistance, be responsible for a secondary infection.

There was no excessive growth of Monilia found on culture even when a selective medium was used. The isolation rate of Monilia in this experiment was slightly higher during the winter examination. As this is the period when an antibiotic therapy is given this could account

for the higher yield at this time. Although Monilia al bicans is usually a saprophytic inhabitant of the respiratory tract it can assume invasive and pathogenic properties.

Until recent years the influence of viral infections in the aetiology of chronic bronchitis did not receive much attention. The predisposing action to infection of the epithelial lesions produced by viral infections has been described (Mulder and colleagues 1952; Hers and Mulder 1953; Straub and Mulder 1948).

The presence of significantly raised antibody titres to influenzae A was demonstrated by Murdoch and colleagues (1959) and has also been observed in the associated virological studies in this investigation. The results found in this study indicate that in this form of investigation a close co-operation between the virologist and the bacteriologist is desirable. In the field of virology it may be that studies on the common cold, a frequent precursor of an acute exacerbation, may yield in formation of great value in the aetiology of chronic bronchitis.

The purpose of the present trial was to see if a continuous antibacterial regime was effective

in preventing exacerbations of bronchial infection in chronic bronchitis.

At the present stage any assessment must of necessity be conjecture. It is of interest that of the twenty seven patients who have remained under observation, ten have produced sputum which has been free from pathogenic organisms since the early part of the trial. It would be gratifying if these patients are receiving therapy and especially if there was an associated clinical response.

SUMMARY

A study of the bacteriology of the sputum of thirty one patients with chronic bronchitis.

Of 31 patients accepted for investigation, 27 have been studied throughout. Approximately half the patients are receiving oxytetracycline but the identity of these cases is not known. Pathogenic organisms were isolated from the sputum of all patients at the start of the trial and are now persisting in seventeen; the other ten have remained clear. The predominant pathogenic organisms isolated were pneumococci and Haemophilus influenzae and of these pneumococci were isolated more frequently. There was no evidence of colonisation of the bronchial tree with coliform bacilli, fungi or staphylococci. No increase in resistance to tetracycline and chloramphenicol was apparent in the Haemophilus influenzae and pneumococci. There was an increased incidence of pneumococci and Haemophilus influenzae during the winter and this has been more marked after a period of fog. The isolation of pneumococci and Haemophilus influenzae

was associated with purulent sputum but there did not appear to be a relationship between the isolation of these organisms and the volume of sputum produced in the morning. Homogenisation of the sputum improved the isolation rate of pathogens. The growth of pneumococci was improved by the addition of carbon dioxide to the atmosphere and also if this was moist. Some pneumococci were isolated which grew only in an atmosphere to which carbon dioxide had been added, these organisms having at the start of the trial grown well without. The presence in the serum of antibodies to Haemophilus influenzae was detected in some patients and additional information received from the virologists indicate that some of the patients have suffered from an influenzal illness at some time.

BACTERIOLOGICAL INVESTIGATION OF PNEUMONIA

This is a report on the bacteriological aspects of an investigation of pneumonia which was undertaken at the City Hospital and the Royal Victoria Hospital. The largest number of patients were investigated at the City Hospital.

The object of the trial was to study as thoroughly as possible the aetiology of pneumonias admitted to these hospitals. The study extended from January 1960 to March 1962.

The type of case admitted to the trial was as follows:-

(1) Patients in whom a diagnosis of pneumonia was made on admission;

(2) Any patient with a temperature of 100°F. or above and in whom a diagnosis of pneumonia was a remotely possible one. The reason for the inclusion of this group was it was considered there are a number of patients who are ultimately diagnosed as virus pneumonias but who are admitted only as unexplained pyrexias.

The number of cases studied was 153. There were 109 male patients and 44 female patients. The patients were adult with the exception of two boys aged 13 and 14 years.

The following bacteriological examinations were done. The sputum was examined on the first, second, fifth and tenth days. When it was not possible to obtain sputum specimens a laryngeal swab was examined instead. Laryngeal swabs were examined from all patients on the first and tenth days. The first day specimens of sputum and laryngeal swabs were taken before any treatment was given and an effort was made by all concerned both in the wards and the laboratory that there was a minimum of delay from the production of the specimen and its treatment in the laboratory.

When the sputum was received at the laboratory it was classified and measured. Three types of sputum were recognised - mucoid, mucopurulent, and purulent. The amount of sputum was recorded by measuring the height of the specimen in a universal container, the result being given in centimetres.

A loopful of the purulent portion of the sputum was inoculated on blood agar and heated blood agar and Gram and Leishman films were made.

After rinsing in saline an equal amount of sterile distilled water and approximately six glass beads were added to the sputum; the specimen was shaken for a minimum of thirty minutes. It was necessary that the total volume did not exceed half the height of the container otherwise homogenisation was impaired and an excess of froth formed.

When the specimen was homogenised, blood agar and heated blood agar plates were inoculated with a loopful of the specimen. Gram and Leishman films were made.

Laryngeal swabs were inoculated on blood agar and heated blood agar. Films were not made.

The inoculated plates were incubated for eighteen hours. At the start of the trial the blood agar plates which had been inoculated with the homogenised specimen of sputum, were incubated in a moist atmosphere which contained 10% carbon dioxide. In the last forty cases two homogenised specimens were examined, one incubated in a normal atmosphere, and the other with the addition of 10% carbon dioxide. This was done in order to test the specific action of carbon dioxide on the growth of pneumococci. The carbon dioxide was produced by the action of hydrochloric acid on chalk. The atmosphere was made moist by the

addition of a jar of water to the container.

Pneumococci were identified by their solubility in 10% sodium desoxycholate, sensitivity to optochin using a disk impregnated with 1:4000 solution, and their ability to ferment inulin using a 1% solution in Hiss's serum water with Andrade's indicator.

Mouse inoculation was practised routinely on the last 420 specimens of sputum. 0.5 ml. of homogenised sputum was inoculated intraperitoneally into a mouse. All specimens of sputum produced by the patients were inoculated into mice.

Typing sera from the State Serum Institute, Copenhagen, were available for the last 114 patients.

Haemophilus influenzae was identified by morphology, colonial appearance and the reaction to Gram stain. Typing sera were available for capsulated strains.

Staphylococcus aureus was identified by morphology, by the golden colour and oil paint appearance of the colonies, and by coagulase production. Facilities were not available for phage typing of the organism.

As soon as possible after admission, a specimen of blood for culture was withdrawn from the patient. This was examined daily and retained for ten days.

A record was kept of all organisms which were found either on culture or in the film whether they were considered to be pathogenic or not. The amount of growth was also noted. This was recorded as ± = a few colonies + = a scanty growth ++ = a moderate growth +++ = a profuse growth. When the smears were examined the organisms and the number of pus cells was recorded in a similar manner. The type of pus cell was identified from the Leishman film.

Determination of the antibiotic sensitivity

When an organism was identified its sensitivity was tested by the disk diffusion method in order to give the physicians this information as soon as possible.

This method was followed by the plate dilution method. It was decided to use heated blood agar as this would sustain the growth of all organisms including Haemophilus influenzae. The sensitivity of pneumococci, Haemophilus influenzae and Kleosiella pneumoniae was tested to the following drugs.

(1) penicillin (2) streptomycin (3) tetracycline (4) chloramphenicol.

The sensitivity of Staphylococcus aureus was tested to:-
(1) penicillin (2) streptomycin (3) tetracycline (4) erythromycin (5) novobiocin (6) chloramphenicol.

The method of the plate dilution method was as follows. The drugs were made up and distributed in the following dilutions (doubling)

Penicillin 0.03 - 8 units/ml
Streptomycin 1.0 - 512 mcg/ml
Tetracycline 0.02 - 256 mcg/ml
Chloramphenicol - 2.0 - 32 mcg/ml
Erythromycin - 0.06 - 1.0 mcg/ml
Novobiocin - 0.5 - 4 mcg/ml

The test organisms were cultured overnight in broth and used undiluted. The inoculum was a loopful using a 3mm platinum loop, and was applied to the surface of the medium. The control organism was a standard sensitive strain of Staphylococcus aureus in broth and was used undiluted after overnight incubation at 37°C. The incubated plates were incubated at 37°C. overnight. The results were expressed as a resistance ratio that is the relationship of the end point of the test strain to the end point of the control strain which is -

$$\frac{\text{end point of test strain}}{\text{end point of control strain}}$$

It was decided to employ the standard Staphylococcus aureus as the control strain as this organism was stable and the maintenance of a stock culture presented no difficulties.

Throughout the investigation there was close co-operation with the virologists who undertook the following examinations.

(1) Serological investigation of all patients.

(2) Virus isolation from patients admitted to one unit participating in the investigation. 90 patients were investigated in this manner.

The viruses investigated were Influenza A, B and C; Adenoviruses; Para influenza 1, 2 and 3; Sendai; Adenoviruses; Psittacosis group viruses; R. burneti; Mumps virus.

Serum was also tested for cold agglutinins.

Results

Pathogenic organisms isolated from sputum and laryngeal swabs.

Number of cases investigated 153

Number of specimens of sputum examined 536

Number of laryngeal swabs examined 285

In table 1 the pathogenic organisms which were isolated on culture for the first time on the several days of examination have been analysed.

Table 1.

Pathogens isolated on the first day of examination.

Pneumococci	from 47 cases
Pneumococci and *Haemophilus influenzae*	from 12 cases
Pneumococci and *Staphylococcus aureus*	from 4 cases
Pneumococci, *Staphylococcus aureus* and *Haemophilus influenzae*	from 5 cases
Pneumococci, *Staphylococcus aureus* and *Klebsiella pneumoniae*	from 1 case
Pneumococci and *Klebsiella pneumoniae*	from 1 case
Staphylococcus aureus	from 19 cases
Staphylococcus aureus and *Haemophilus influenzae*	from 2 cases
Haemophilus influenzae	from 14 cases
Pneumococci, Haemophilus influenzae and *Klebsiella pneumoniae*	from 1 case

Organisms isolated on the second day when no pathogens were isolated on the first day

Staphylococcus aureus	from 6 cases

Organisms isolated on the fifth day when no pathogens were isolated on previous examinations.

Pneumococci	from 2 cases
Staphylococcus aureus	from 5 "

Organisms isolated on the tenth day when no pathogens were isolated previously.

Pneumococci	from 1 case
Klebsiella pneumoniae	" 1 case
Staphylococcus aureus	" 3 cases
Haemophilus influenzae	" 1 case

Organisms isolated on the second day when other pathogens were isolated on the first day.

Staphylococcus aureus	from 1 case
Haemophilus influenzae	" 4 cases

Organisms isolated on the fifth day when other pathogens were isolated previously.

Klebsiella pneumoniae	from 1 case
Haemophilus influenzae	" 4 cases
Staphylococcus aureus	" 3 cases

Organisms isolated on the tenth day when other pathogens were isolated previously.

Pneumococci	from	1 case
Klebsiella pneumoniae	"	1 case
Staphylococcus aureus	"	8 cases

First Day Examination

It can be seen that pneumococci were predominant and were isolated from 71 cases. A pure culture was obtained in 47 cases. In the remaining 24 cases the most commonly associated organism was Haemophilus influenzae. Second in frequency of isolation was Haemophilus influenzae. In 14 cases a pure culture was obtained and in the remaining 20 cases it was isolated together with other organisms. Staphylococcus aureus was isolated from 31 cases, in pure culture in 19 cases and with other organisms in 12 cases. Klebsiella pneumoniae was isolated infrequently. In the three cases where it was found there was also a growth of other organisms.

Second Day Examination

Staphylococcus aureus was isolated from 7 cases. In six cases no pathogenic organism had been isolated on the first day of examination.

A growth of Haemophilus influenzae was obtained in 4 cases where another pathogen had been isolated on the first day.

Fifth day examination.

There were two isolations of pneumococci in cases where no pathogen had been isolated previously. Staphylococcus aureus was isolated from 8 cases. In five of these no pathogenic organism had been found on earlier examination. There were 4 isolations of Haemophilus influenzae and 1 of Klebsiella pneumoniae from cases where no pathogenic organisms had been found on previous examination.

Tenth Day Examination

There were 11 isolations of Staphylococcus aureus. 8 of these were from cases where no pathogen had been found on earlier examination. Pneumococci were isolated from 2 cases. In one of these no other pathogen had been found previously. Klebsiella pneumoniae was found in 2 cases. No pathogen had been found previously in one of these cases. There was one isolation of Haemophilus influenzae from a case where other organisms had been obtained at earlier examination.

It is of interest that in nineteen cases Staphylococcus aureus was isolated for the first time on the fifth or tenth day of the pneumonia. The growth of organisms in these cases was classified as scanty or moderate and never was profuse. The isolation of six organisms was not considered of clinical significance and did not lead to a worsening of the patient's condition.

In addition to these primary isolations, in several cases pathogenic organisms were isolated on more than one examination.

Table 2.

Day of examination on which organisms were isolated.	The number of cases and the pathogenic organisms isolated.			
	Pneumococci	Staph. Aureus	Haemo. influenzae	Kleb. pneumoniae
1st and 2nd.	12	11	4	1
1st, 2nd & 5th	1	2	1	0
1st, 2nd & 10th	4	2	1	0
1st and 5th	1	2	1	2
1st, 5th and 10th	1	2	0	0
1st and 10th	7	2	5	0
5th and 10th	0	2	0	1
2nd and 10th	0	2	0	0
	26	25	11	4

Organisms were isolated from the largest number of cases on the first and second days. Pneumococci were isolated from twelve cases, Staphylococcus aureus from eleven cases, Haemophilus influenzae from four cases and Klebsiella pneumoniae from one case on these two days of examination. In the majority of these cases the organisms were not isolated at later examinations. There were some cases where organisms were isolated at early and late examinations, for example in seven cases pneumococci were isolated on the first and the tenth days.

In order to determine the significance of strains of organisms isolated at later examinations, it is necessary where possible to identify the type present. In the present study phage typing of staphylococci was not undertaken and typing sera for Klebsiella were not available; it was found that all strains of Haemophilus influenzae isolated were unencapsulated. Typing sera were available for some strains of pneumococci and it was possible by this means to identify some organisms isolated at more than one examination. Three of the strains isolated on the first and second days were identified as type 3 pneumococci, one strain isolated on these

days was identified as a type 12 pneumococcus, and one other as a type 8 pneumococcus. Two strains isolated on the first and fifth days were identified, one was a type 20 pneumococcus and the other a type 32 pneumococcus. A type 3 pneumococcus was isolated from one patient on the first and tenth days of examination. The isolation of pneumococci particularly virulent strains, on the first and second days might appear to indicate a severe degree of infection which was not immediately controlled by treatment. Where however organisms were isolated at late examination and were not associated with exacerbations of infection as in the present series, the possibility of contamination from the throat should be considered.

The preceding analysis is a purely bacteriological one. When the bacteriological findings were considered in relation to the clinical findings found on admission, the cases were classified in the following groups.

Infecting Agents

Pneumococcus	in 48 cases
Pneumococcus and Haemophilus influenzae	in 20 cases
Haemophilus influenzae	in 15 cases
Staphylococcus aureus	in 11 cases
No specific pathogenic bacteria isolated	in 59 cases
	153

Relation of age and sex of patient to type of bacterial infection

In table 3 the age and sex of the patients and the type of bacterial infection have been analysed.

Table 3.

Age	Staph. aureus		Haemophilus influenzae		Pneumo.& Haemophilus influenzae		Pneumococci		No paths	
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.
0-20	0	0	2	1	0	1	0	0	4	1
20-30	0	0	0	0	0	1	3	1	3	2
30-40	4	0	1	2	0	1	3	1	0	3
40-50	1	0	2	0	3	0	5	2	3	2
50-60	0	0	1	1	2	0	12	2	5	2
60upwards	6	0	3	2	9	3	15	4	22	12

There was a larger number of male than female patients. 109 patients were male and 44 were female. There was however a larger number of beds available for male patients. There was a predominance of patients of both sexes in the higher age groups. 76 of the males (69%) and 26 of the females (59%) were over the age of 50 years.

There was an increased isolation of pneumococci in males over 50 years of age as compared with the younger patients. Of 38 cases where a pure culture of pneumococci was obtained, 27 (or 71%) were men over 50 years of age. Where a combined infection with pneumococci and Haemophilus was considered responsible 78% of these isolations were from males over 50 years of age.

In male patients, over 50% of the staphylococcal infections were found in patients over 60 years of age. Staphylococcus aureus was not considered as the pathogen responsible for infection of any of the female patients.

There was a failure to isolate pathogenic bacteria from a higher proportion of the older patients as compared with the younger age group. Of the 37 male cases which were not diagnosed bacteriologically, 27 i.e. 73% were over the age of 50 years. Of the female patients in this group, 14 out of 22 i.e. 63% were over the age of 50 years.

The general conclusion from this analysis was that in male patients there was a higher isolation rate of pneumococci with increasing age, and also a tendency for no specific bacterial pathogen to be found in the older patients both male and female.

Pneumococcal Types isolated during the investigation

Pneumococcal types in relation to severity of disease and age of patient and radiological evidence of pneumonia.

In table 4 the pneumococcal types have been analysed in relation to the age of the patient, the severity of the pneumonia, and the radiological diagnosis of the pneumonia.

Table 4.

Patient No.	Pneumococcal type	Age	Radiological diagnosis	Severity
1	1	55	Segmental	Moderate
2	1+	64	Lobar	Severe
3	3	43	No change	Moderate
4	3+o	60	Lobar	Severe
5	3+o	79	Lobar	Severe
6	3+o	66	Lobar	Severe
7	3+	56	Lobar	Severe
8	3	64	Lobar	Severe
9	3	33	Segmental	Moderate
10	3	23	Lobar	Moderate
11	3o	70	Lobular	Severe
12	3	57	Lobular	Moderate
13	3	56	Lobular	Moderate
14	4	47	Lobar	Severe
15	5	74	Lobar	Moderate

TABLE 4 (cont)

Patient No.	Pneumococcal type	Age	Radiological diagnosis	Severity
16	5+	34	Lobar	Severe
17	6o	88	Lobular	Severe
18	8	30	Lobar	Moderate
19	8	38	Lobar	Moderate
20	8	48	Segmental	Moderate
21	9	61	No change	Moderate
22	9	84	Lobar	Moderate
23	10	69	Lobular	Moderate
24	11	47	Lobular	Moderate
25	12	69	Segmental	Moderate
26	14+o	82	Segmental	Severe
27	14	88	Lobular	Moderate
28	20	75	Segmental	Moderate
29	21	60	Segmental	Moderate
30	23+	75	Lobar	Moderate
31	23	50	No change	Moderate
32	23	80	Segmental	Moderate
33	32	46	Segmental	Moderate
34	35	47	No change	Moderate
35	35	62	Lobular	Severe

+ Bacteraemia present
o Fatal case

35 types were identified. 11 strains belonged to type 3 which is 31% of the total. In this group there were four deaths and in three of these cases there was associated bacteraemia. All these patients were aged 60 years and over.

In table 5 the cases from which type 3 pneumococci were isolated have been analysed according to the severity of the illness and the age of the patient.

Table 5.

Age	Moderate	Severe
0-30	1	0
30-40	1	0
40-50	1	0
50-60	2	1
60-70	0	3
70 upwards	0	2

Six cases were classified as severe and five as moderate. The table shows that there was a tendency for the severity of the illness to increase with the rising age of the patient.

In table 6 the types have been divided into two groups.

1. Types 1-8 which are considered the more virulent types.
2. Type 9 and upwards. Again they have been considered in/

in relation to the severity of the illness and the age of the patient.

Table 6.

Age	Types 1-8 (57%)		Types 9 and upward (43%)	
	Moderate	Severe	Moderate	Severe
0-30	1	0	0	0
30-40	3	1	0	0
40-50	2	1	3	0
50-60	3	1	1	0
60-70	0	4	4	1
70 upwards	1	3	5	1

19 of the strains of pneumococci which were typed were obtained from patients over 60 years of age. Of these, 8 belonged to types 1-8 and this included 5 strains of type 3 pneumococci. The remaining 11 strains belonged to the higher types and in nine cases infected by these types, the illness was of a moderate nature.

In the patients under 60 years of age 12 out of the 16 patients in this age group were infected with types 1 to 8. The illness in 9 of these cases was regarded as moderate in nature.

Radiological findings in relation to type of pneumococcus isolated

On radiological evidence the cases were divided into three main groups, lobar pneumonia, lobular pneumonia, and segmental pneumonia. There were also some cases where there was no radiological evidence of pneumonia.

In table 7 the cases have been analysed according to the type of pneumonia diagnosed radiologically and the infecting type of pneumococcus. The pneumococcal types have been divided into two groups, those of the lower types 1-8 and those above that number. As lobar and segmental indicate degrees of consolidation these pneumonias have been grouped together.

Table 7.

	Lobar and Segmental	Lobular	No evidence of pneumonia
Types 1-8	15	4	1
Types 9 & upwards	8	4	3

It can be seen that the lower types were responsible for the largest number of cases of lobar and segmental pneumonia. However in just over half the cases due to infection with the higher types, the pneumonia was either lobar or segmental in character.

The conclusions which may be drawn from this study are

1. The younger patients were more commonly infected with the lower types of pneumococci which in most of these patients caused a moderate illness.
2. Older patients when infected with the lower types of pneumococci tended to have a severe illness. This was particularly marked when the infecting type was type 3.
3. The higher types of pneumococci were isolated more frequently from the older patients and the illness was generally of a moderate nature.
4. Of the cases where the infecting pneumococcus belonged to the lower types the largest proportion of cases (75%) were lobar or segmental in type.
5. Of the cases here the infecting type of pneumococcus belonged to the higher types, 53% were lobar or segmental in type.

Bacteraemia

A growth of pneumococci was obtained on culture of the blood of ten patients in the investigation. No other organisms were found by this method of examination.

This is a percentage of 0.65% of all cases in the study or 14.7% of cases where the infecting organism was the pneumococcus.

As typing sera were not available at the start of the trial, only eight strains were typed.

The identified types and the ages of the patients have been listed below.

Type	Age
1	64
3	66+
3	60+
3	56
3	79+
5	34
14	82+
23	75
Not typed	71
Not typed	61

+ Fatal case

Type 3 was the predominant type and three of the cases where this was the infecting type had a fatal outcome. The other patient who died was infected with a type 14 pneumococcus.

All these four patients were over 60 years of age, as were most of the patients in whom bacteraemia was present. The high incidence of bacteraemia in older patients is seen in the 25.8% incidence of this condition in patients with pneumococcal pneumonia over 60 years. Bacteraemia was present in 5.4% of such patients under the age of 60 years.

From the present study it would appear that bacteraemia was a serious complication of pneumococcal pneumonia in elderly people, as seen by the high proportion of deaths which occurred in this group.

Two strains of pneumococci, a type 14 and a type 23, were isolated from the blood in two cases where sputum specimens were not available. In these cases culture of laryngeal swabs did not yield a growth of pneumococci.

Mouse Inoculation

Intraperitoneal inoculation of sputum

420 specimens of sputum were inoculated intraperitoneally into mice. The pathogenic organisms obtained by this technique can be seen on table 8.

Table 8

Analysis of pathogens isolated

Pathogens isolated	1st day	2nd day	5th day	10th day
Pneumococci	34	7	1	1
Staphylococcus aureus	2	2	0	1
Klebsiella pneumonia	0	0	1	0
Haemophilus influenzae	2	1	0	0

Fifty two pathogens were isolated by mouse inoculation. The most frequently isolated pathogen was the pneumococcus which was present on forty three occasions. The largest number of organisms was isolated at the start of the pneumonia, although there were single isolations of Staphylococcus aureus, Klebsiella pneumoniae and pneumococci on the tenth day.

On fourteen occasions mouse inoculation was the only effective method of isolation of pneumococci from the sputum. Twelve of these were on the first day, one on the second day, and one on the fifth day.

Pneumococcal types isolated by mouse inoculation only

On the first day

Type 1	1 strain
Type 3	3 strains
Type 4	1 strain
Type 5	1 strain
Type 8	1 strain
Type 9	1 strain
Type 10	1 strain
Type 14	1 strain
Not typed	2 strains

Isolated on the second day

1 strain which was not typed.

Isolated on the fifth day

Type 3	1 strain

It can been seen that the types isolated only by mouse inoculation were mostly those considered to be of a virulent nature.

Pneumococci isolated from the sputum which were not mouse virulent

Pneumococcal types isolated from the sputum which were not mouse virulent were as follows.

Type 12	1 strain
Type 21	1 strain
Type 32+	1 strain
Type 35	2 strains

+ This strain was isolated on the fifth day. On the first day a type 32 mouse virulent pneumococcus was isolated from the sputum.

It can be seen that strains of the lower types possessed the greater degree of mouse virulence. Absence of this property in higher types did not necessarily result in a less severe infection in man, as seen in a severe pneumonia caused by infection with a type 35 pneumococcus.

Patients from whom no bacterial pathogens were isolated

Fifty nine patients were in the group where no significant pathogenic bacteria were isolated at the start of the illness.

Thirty one patients had received antibiotic treatment before admission, twenty five had not received treatment and in three cases no information was available.

In seven of the 31 cases who had received previous treatment, there was serological evidence of a virus infection. Four cases showed a rise in antibody titre, and three had raised titres which were considered suggestive of a recent infection.

Analysis of the twenty eight cases where no known treatment had been given

Presence of a viral infection.

Four cases were considered to have a current infection. In three cases this diagnosis was based on serological evidence of a rise in virus antibodies. From one patient a virus was isolated.

Three patients were regarded as having had a recent infection on serological evidence of significantly raised antibody titres.

It was considered that these infections were the possible cause of the pneumonia.

There remained twenty one cases where no previous treatment had been given and there was no evidence of a viral infection.

These cases have been analysed in more detail.

Age distribution of remaining twenty one cases

Age groups	No pathogen group	Other patients in trial
	Number of cases	Number of cases
0-30	4	15
30-40	1	14
40-50	2	16
50-60	1	24
60-70	6	39
70-80	3	14
over 80	4	10

The majority of patients (62%) were over 60 years of age which is in accordance with the general age distribution of the patients in the trial. There was however a higher percentage of patients over 80 years in age in this group, 19%, as compared with 7.5% of such patients in the remainder of the series.

Bacteriological findings in the 21 cases.

A profuse growth of Monilia was obtained in two cases, and a profuse growth of Bact.coli in one case. In three cases there was a moderate growth of Staphylococcus aureus and from one case a growth of Proteus.

These organisms were not regarded as of clinical importance. The organisms isolated from the remaining cases were N. catarrhalis and Strept. viridans. This was the bacterial flora of all cases over 80 years of age.

The type of specimen examined

No sputum specimens were received from four patients on the first day of examination. In these cases laryngeal swabs were examined. Mouse inoculation was therefore not done in these cases. In two cases the sputum specimen received was salivary in type.

Summary of findings

59 cases were not diagnosed bacteriologically. 31 cases had received previous antibiotic treatment. In 7 of these it was considered that a virus may have been the cause of infection.

In 7 of the remaining 28 cases it was considered that a virus was a possible cause of infection.

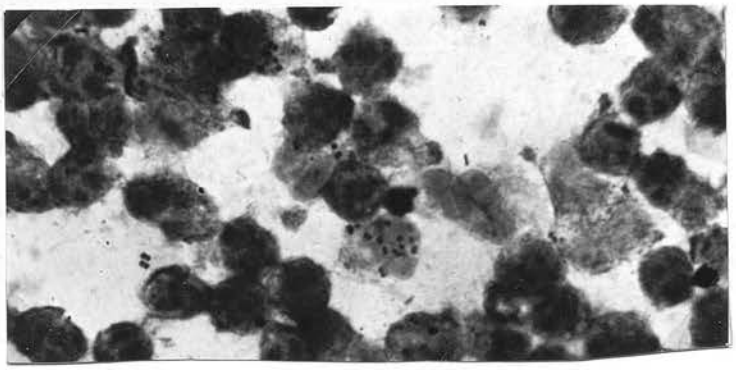
Although bacterial pathogens were isolated from some of the remaining 21 cases it was not considered that they were the cause of the pneumonia.

In 6 other cases a less complete examination was done, as only laryngeal swabs were received from four patients and in 2 cases the sputum specimens were salivary in nature and not considered satisfactory.

'Non-pathogenic' organisms isolated during investigations

A record was made of all organisms isolated in the investigation. The non pathogenic organisms most frequently isolated were N. catarrhalis and Strept. viridans which were generally found with pathogenic bacteria at the start of the pneumonia, and remained in the sputum after the infecting organisms had disappeared. In most cases the presence of N. catarhalis and Strept.

Film of sputum from patient with pneumonia, showing intracellular neisseriae. No bacterial pathogens were isolated and there was evidence of a previous viral infection.



viridans alone was not associated with purulence of the sputum.

There were two exceptions. One patient who showed serological evidence of a recent viral infection, produced a purulent sputum with numerous neisseriae on culture on the first day. Examination of a Gram film showed numerous neisseriae many of which were intracellular in distribution, indicating an acute neisserial infection. This film is illustrated on page 257.

The second patient also had serological evidence of a recent viral infection and very purulent sputum. Culture of this specimen yielded a profuse growth of Strept. viridans and large numbers of streptococci were observed in a smear stained by Gram's method.

Monilia and coliform bacilli isolated at later examinations.

These organisms were isolated from several patients at later examinations, i.e. the fifth and tenth days.

Monilia	from	19	patients
Bact.coli	"	31	"
Proteus	"	3	"
Ps. pyocyanea	"	3	"
Klebsiella pneumoniae	"	2	"
Monilia and Bact.coli	"	3	"
Monilia and Ps. pyocyanea	"	1	"
Monilia and Klebsiella pneumoniae	"	1	"

Three strains of Bact. coli and one strain of Ps.pyocyanea were mouse virulent.

With the exception of Ps. pyocyanea, the growth obtained on culture of these organisms was not excessive, and there was generally an associated growth of N. catarrhalis and Strept. viridans. Ps. pyocyanea grew profusely and in pure culture; it was considered of possible significance in only one patient. This patient who was initially infected with a type 3 pneumococcus died, and Ps. pyocyanea was isolated from tracheostomy specimens in the terminal stages of the illness.

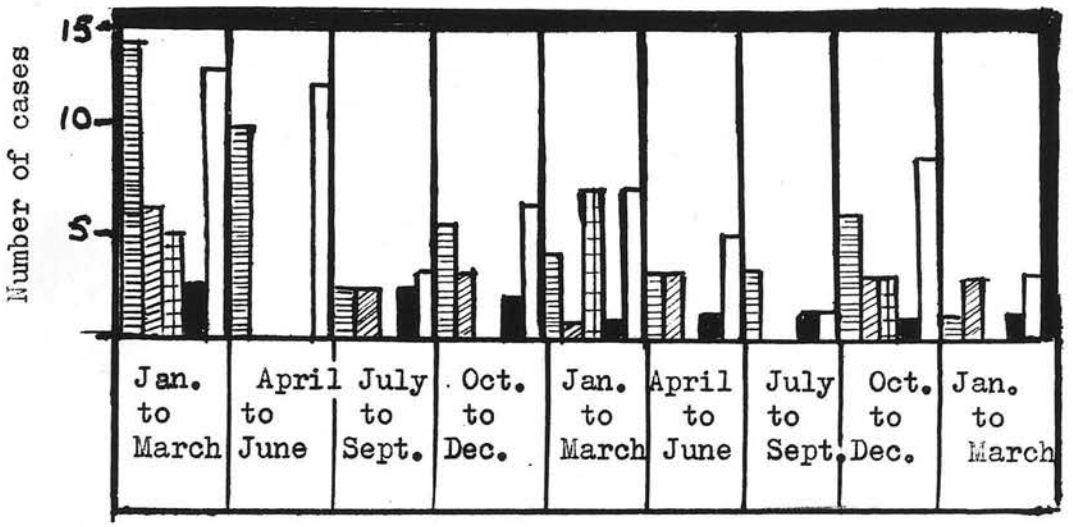
Seasonal incidence (Figure 1 on page 261.)

This analysis does not indicate the number of cases of pneumonia admittedto hospital, as for various reasons some patients suffering from pneumonia were not placed in the trial. In

addition it cannot be regarded as an indication of the number of cases of pneumonia in Edinburgh and district during the time of investigation. These admissions were dependent on the number of empty beds which were available.

It does give an indication of the distribution of organisms throughout the years, although the numbers in each group are small. As would be expected the highest isolation of pathogenic organisms was during the winter months. There was a high isolation of rate of pneumococci in the Spring of 1960, which is associated with the tendency for lobar pneumonia to occur in this season. All organisms with the exception of pure growths of Haemophilus influenzae were found throughout the year. Haemophilus influenzae in pure culture was isolated only in the winter months. The pneumonias from which no specific bacterial agent was isolated occurred throughout the year. There did not appear to be a marked seasonal incidence of this type of case.

Figure 1



Seasonal incidence of pneumonias, January 1960 to March 1962.

Infecting agents

Pneumococcus

Pneumococcus and H. influenzae

H. influenzae

Staphylococcus aureus

No specific pathogenic bacteria isolated.

Factors which influenced the isolation rate of pathogenic organisms.

Comparison of isolation rate of organisms from homogenised sputum and untreated sputum

Pneumococci

Sixteen strains of pneumococci out of a total of ninety one isolated from the sputum, were isolated from the homogenised specimen but were not obtained from the untreated specimen of sputum. On no occasion was the organism isolated from the untreated specimen and not found in the homogenised one.

Staphylococcus aureus.

Ten strains out of a total of seventy seven were isolated from the homogenised specimen and were not obtained from the untreated one. On no occasion was the organism isolated from the untreated specimen and not found in the homogenised one.

Klebsiella pneumoniae

All strains of Klebsiella pneumoniae - a total of eight were obtained on culture of both the homogenised and the untreated specimen.

Haemophilus influenzae

There were forty five isolations of Haemophilus influenzae from the sputum.

In this comparison the effect of both homogenisation and culture on heated blood agar are considered.

Isolation from untreated sputum

Cultured on blood agar 25

Cultured on heated blood agar 35

Isolation from homogenised sputum

Cultured on blood agar 35

Cultured on heated blood agar 45

It can been seen that with the exception of Klebsiella pneumoniae homogenisation of the sputum resulted in a higher isolation rate of pathogenic organisms. For the isolation of Haemophilus influenzae culture of homogenised sputum on a selective medium was the most effective method of isolation of this organism.

The effect of carbon dioxide on the growth of pneumococci.

The addition of carbon dioxide enhanced the growth of all strains of pneumococci which were cultured under its influence.

There were 2 strains out of 92 strains isolated from culture of sputum and blood and by mouse inoculation which grew only in the presence of carbon dioxide. These strains were identified as type 8 and type 3 pneumococci. They were mouse virulent and it was observed that the strains isolated by animal inoculation required the same

cultural conditions. Both patients from whom these strains were isolated were classified as severe chronic bronchitics.

The effect of a moist atmosphere on the growth of pneumococci.

Culture in a moist atmosphere was beneficial to the growth of pneumococci, the colonies being larger and more mucoid in nature.

Anaerobic culture.

This was not done as a routine procedure. One strain, a type 23 pneumococcus which was seen in large numbers in the Gram film, but which did not grow on aerobic culture was found to be an obligatory anaerobe.

Comparison of isolation rate of pathogenic organisms from sputum and laryngeal swabs.

These specimens were examined on the first and tenth day of the investigation. The results have been analysed in table 9.

Table 9.

Number of pathogens isolated on culture of a laryngeal swab but which were not found on culture of the sputum on the first day.

Pneumococci	4 strains
Staphylococcus aureus	7 strains
Haemophilus influenzae	3 strains

On the tenth day

Staphylococcus aureus	3 strains
Haemophilus influenzae	1 strain
Klebsiella pneumoniae	1 strain

Number of pathogens isolated on culture of the sputum but which were not isolated on culture of a laryngeal swab.

On the first day

Pneumococci	36 strains
Staphylococcus aureus	9 strains
Haemophilus influenzae	23 strains
Klebsiella pneumoniae	1 strain

On the tenth day

Pneumococci	8 strains
Haemophilus influenzae	6 strains
Staphylococcus aureus	3 strains
Klebsiella pneumoniae	4 strains

It can be seen clearly that culture of the sputum was the more effective method of isolation of pathogenic bacteria.

Forty four strains of pneumococci isolated from sputum were not cultured from laryngeal swabs examined on the same day. There were also twenty nine strains of Haemophilus influenzae which were found only on culture of the sputum. Staphylococcus aureus appeared to be missed with amost equal freqency from both sputum specimens and laryngeal swabs.

Although culture of the sputum gave better results there were occasions when culture of a laryngeal swab was the only effective method of isolation of pathogenic organisms.

Identification of pneumococci by bile solubility, optochin sensitivity and inulin fermentation.

Organisms were identified as pneumococci when the following criteria were satisfied.

1. Culture on blood agar; round smooth colonies with a raised outer edge.
2. An agranular growth in serum broth.
3. Alpha haemolysis on heated blood agar.
4. Demonstration of capsules.

a. Before typing sera were available, capsules were demonstrated in dry India ink films.

b. When typing sera were available, by the identification of the specific serotype.

5. Solubility in bile, using 10% sodium desoxycholate.
6. Sensitivity to optochin using disks impregnated with 1:4000 solution.
7. Fermentation of inulin using 1% solution of the sugar in Hiss's serum water.

An association was observed between the sensitivity of the pneumococcus to penicillin and the possession by the organism of the accepted biochemical reactions of bile solubility, optochin sensitivity and inulin fermentation. The sensitivity to penicillin of the strains of pneumococci isolated are detailed on pages 270-272.

It was observed that when the resistance ratio to penicillin was one and under the great majority of strains possessed the properties of bile solubility, optochin sensitivity, and inulin fermentation.

There were a few exceptions and details of these are appended.

Bile Solubility

Four strains of total of 115 strains isolated from blood, sputum and by mouse inoculation appeared to be bile insoluble when

tested with 10% sodium desoxycholate. In all the tests there was some degree of clearing, but it was considered these strains could not be termed completely bile soluble.

Optochin sensitivity

Five strains out of 115 strains isolated from blood, sputum and by mouse inoculation were optochin resistant.

Inulin fermentation.

Seven of the 115 strains isolated from blood, sputum and by mouse inoculation failed to ferment inulin.

Four of the above strains lacked the two properties of optochin sensitivity and inulin fermentation.

When biochemical reactions of organisms morphologically and colonially resembling pneumococci, with a penicillin resistance ratio of two and over were tested, it was found that all such organisms, a total of 33 were insoluble in 10% sodium desoxycholate and insensitive to optochin. The property of inulin fermentation was present in four and absent in the remaining twenty nine strains.

Examination of Leishman film.

536 films were examined. Six films of

sputum from pneumonia on the first day showed an excess of eosinophils. In these cases films of sputum obtained during the later examinations did not show an excess of eosinophils.

Antibiotic sensitivity

The method used was the plate dilution method. The medium employed was heated blood agar. As the control organism, a standard sensitive strain of Staphylococcus aureus was used. Both the control organism and the test organisms were incubated at 37°C overnight in broth and used undiluted. The inoculated plates were incubated at 37°C overnight.

The results were expressed as a resistance ratio, that is the relationship of the end point of the test strain to the end point of the control strain which is -

$$\frac{\text{end point of test strain}}{\text{end point of control strain}}$$

The organisms studied have been divided into three groups

(1) those isolated from sputum and laryngeal swabs

(2) those isolated by mouse inoculation and

(3) those isolated by culture of the blood.

Pneumococci

Sensitivity to penicillin

The standard sensitive Staphylococcus aureus was inhibited by a concentration of penicillin which ranged from 0.12 to 0.25 units per ml.

Pneumococci isolated by direct culture of sputum or laryngeal swabs

The results have been summarised on table 10.

Table 10

Resistance ratios

Days of examination.	1 & under	2	4	8 & over
1st	54	3	0	4
2nd	9	1	4	1
5th	2	0	2	2
10th	6	0	1	6

It can be seen that most of the strains isolated on the first and second days had a ratio of one and under. Seven strains were isolated on the first day which had a ratio of two and over and in four of these the ratio was eight and over. Strains with this ratio were isolated on the second, fifth and tenth days. The largest

number of strains with the greatest degree of resistance were found on the tenth day. Not all the strains isolated on the fifth and tenth days had ratios which suggested resistance to the drug; eight of the nineteen strains isolated on these days had a ratio of one or under.

Pneumococci isolated by inoculation of mice with sputum.

The results have been summarised in table 11.

Table 11.

Days of examination	Resistance Ratios: 1 & under	2	4	8 & over
1st	30	2		2
2nd	5		1	1
5th			1	
10th			1	

It can be seen that most of the strains isolated had a ratio of under one or two. Six strains were isolated with a ratio of four and over. Two of these were found on the first day of examination and four on the second, fifth and tenth days.

Pneumococci isolated from the blood.

Ten strains of pneumococci were cultured from the blood. Nine of these gave a ratio of one or under. The remaining strain which was a type 3 pneumococcus, gave a ratio of over eight.

Sensitivity to tetra cycline

The standard sensitive Staphylococcus aureus was inhibited by a concentration of tetracycline which ranged from 2 to 4 mcg per ml.

Pneumococci isolated by direct culture of sputum or laryngeal swabs.

The results have been summarised in table 12.

Table 12.

Days of examination	Resistance Ratios: 1 & under	2	4	8
1st	46	6	9	
2nd	8	3	4	
5th	1		4	1
10th	4	3	6	

Fifty nine strains had a ratio of one or under, twelve a ratio of 2, twenty three a ratio of four and one a ratio of eight. Apart from the strain isolated on the fifth day, with a ratio

of eight, there was not an obvious trend for strains with a high ratio to be found at later examination.

Pneumococci isolated by mouse inoculation

The results have been summarised in table 13.

Table 13.

Days of examination	Resistance ratios: 1 & under	2	4	8 & over
1st	30	3	1	
2nd	6	1		
5th	1			
10th			1	

There were thirty seven strains with a ratio of one or under, four with a ratio of two and two with a ratio of four. The one strain isolated on the tenth day had a ratio of four.

Pneumococci isolated from blood

Ten strains of pneumococci were cultured from the blood. Nine had a resistance ratio of one or under and one a resistance ratio of four. This last strain was a type 3 pneumococcus.

It was possible to identify the pneumococcal type of eight strains which had a ratio of four or over.

These were as follows:-

Type 3	three strains
Type 6	one strain
Type 12	one strain
Type 23	one strain
Type 32	one strain
Type 35	one strain

Sensitivity to streptomycin

The standard sensitive Staphylococcus aureus was inhibited by a concentration of streptomycin which ranged from 2 to 4mcg. per ml.

Pneumococci isolated by direct culture of sputum or laryngeal swabs.

The results have been summarised in table 14.

Table 14

Days of examination	Resistance ratios 1	2	4	8&over
1st	21	8	2	30
2nd	4	0	0	11
5th	0	0	2	4
10th	0	0	0	15

Thirty three strains had a resistance ratio of two and under. Sixty four strains had a

resistance ratio of four or over. Of these, thirty two were isolated on the first day, and eleven on the second day. All twenty one strains isolated on the fifth and tenth days had a resistance ratio of four or over.

Pneumococci isolated by mouse inoculation

The results have been summarised on table 15.

Table 15

Days of examination	Resistance ratios 1	2	4	8&over
1st	13	3	0	18
2nd	4	0	0	3
5th	0	0	1	0
10th	0	0	0	1

There were twenty three strains with a resistance ratio of four and over and twenty with a resistance ratio of two and under. Of the strains isolated on the first and second day, approximately half had a resistance ratio of two or under, and half a resistance ratio of eight or over. Two strains were isolated on the fifth and tenth days. They had resistance ratios of four and eight respectively.

Pneumococci isolated from blood

Ten strains of pneumococci were cultured from the blood. Five had a resistance ratio of one or under, and five a resistance ratio of eight or over.

There was a varied distribution of pneumococcal types which showed resistance to streptomycin.

Four of the strains isolated from the blood were identified. These were two type 3, one type 23 and one type 14. Streptomycin resistant strains isolated from the sputum were as follows. One type 3, one type 11, one type 12, one type 20, one type 21, one type 32 and one type 35.

With the exception of the type 14 and type 23 pneumococci, all strains isolated from the blood were also found in the sputum and all were resistand to streptomycin.

Chloramphenicol

The standard sensitive Staphylococcus aureus was inhibited by a concentration of chloramphenicol of 8 mcg per ml.

All strains of pneumococci isolated during the trial were sensitive to chloramphenicol with ratios of under one.

Staphylococcus aureus

Sensitivity to penicillin

The standard sensitive Staphylococcus aureus was inhibited by a concentration of penicillin which ranged from 0.12 to 0.25 units per ml.

Staphylococcus aureus isolated by direct culture of sputum or laryngeal swab.

The results have been summarised in table 16.

Table 16.

Days of examination	Resistance ratios: 1& under	2	4	8& over
1st	9	1		19
2nd	9	1		13
5th	1	1		12
10th		1		20

The organisms were in two main groups, a larger group where the resistance ratio was eight and over, and a smaller group where the ratio was two and under. 62% of the strains isolated on the first and second days had a ratio of eight and over. Nineteen of the thirty two strains isolated on the fifth and tenth days which had a ratio of eight or over, were isolated for the first time on these days of examination. This

might be considered as evidence of spread of penicillin resistant strains in the hospital environment. As phage typing of staphylococci was not undertaken and nasal swabbing not performed, the source and spread of these organisms in this investigation cannot be accurately determined.

Staphylococcus aureus isolated by mouse inoculation

Five strains were isolated. Two were isolated on the first day, two on the second day, and one on the tenth day. All strains had a resistance ratio of eight or over.

Sensitivity to tetracycline

The standard sensitive Staphylococcus aureus was inhibited by a concentration of tetracycline which ranged from 2 to 4 mcg per ml.

Staphylococcus aureus isolated by direct culture of sputum or laryngeal swab.

The results have been summarised in table 17.

Table 17

Days of examination	Resistance ratios: 1&under	2	4	8
1st	17	3		9
2nd	9	5		9
5th	3	1		10
10th	5	2		14

Forty five strains had a resistance ratio of two or under and forty two a resistance ratio of eight or over. There was a tendency for higher resistance ratios to be associated with the later examinations. Of thirty five strains isolated on the fifth and tenth days, twenty four had a resistance ratio of eight or over and this included nineteen strains isolated for the first time on these days.

Staphylococcus aureus isolated by mouse inoculation.

The results have been summarised in table 18.

Table 18

Days of examination.	Resistance ratios: 1 & under	2	4	8 & over
1st	2			
2nd	1			1
5th				
10th				1

Five strains were isolated by mouse inoculation. Three isolated on the first and second day had a resistance ratio of one or under. Two strains isolated on the second and tenth days had resistance ratios of eight or over.

Sensitivity to streptomycin

The standard sensitive Staphylococcus aureus was inhibited by a concentration of streptomycin which ranged from 2 to 4 mcg per ml.

Staphylococcus aureus isolated by direct culture of sputum or laryngeal swabs.

The results have been summarised in table 19.

Table 19

Resistance ratios

Days of examination.	1 & under	2	4	8 & over
1st	19	3		7
2nd	11	1		11
5th	3	1		10
10th	5	2		14

There were two groups of organisms, those with a resistance ratio of two and under and those with a resistance ratio of eight and over. There were thirty eight in the first group and forty two in the second group. There was a tendency for organisms isolated on the fifth and tenth days to have an increased resistance ratio. Of thirty five strains isolated on these days twenty four had a resistance ratio of eight or over, and this included nineteen strains isolated for the first time on these days.

Staphylococcus aureus isolated by mouse inoculation.

Five strains were isolated. Three had a resistance ratio of one or under. Two of these were isolated on the first day and one on the second day. Two strains had a resistance ratio of eight or over. One was isolated on the second day and one on the tenth day.

Sensitivity to chloramphenical

The standard sensitive Staphylococcus aureus was inhibited by a concentration of chloramphenicol of 8 mcg. per ml.

All strains of Staphylococcus aureus isolated during the trial had resistance ratios of under one.

Sensitivity to novobiocin

The standard sensitive Staphylococcus aureus was inhibited by a concentration of novobiocin of 2 mcg. per ml.

All strains of Staphylococcus aureus isolated during the trial had resistance ratios of under one.

Sensitivity to erythromycin

The standard sensitive Staphylococcus aureus was inhibited by a concentration of erythromycin of 0.12 mcg. per ml.

With the exception of one, all strains of Staphylococcus aureus isolated during the trial had resistance ratios of one and under one.

The strain which showed increased resistance was isolated on the twelth day from the sputum of a patient with a staphylococcal pneumonia treated with erythromycin. Strains which were inhibited by a concentration of 0.12 mcg. per ml. were isolated on the first, second, fifth and tenth days. The inhibitory concentration of the drug for the organism isolated on the twelfth day was 8 mcg. per ml. and this level remained at subsequent examinations.

Haemophilus influenzae

Sensitivity to penicillin

The standard sensitive Staphylococcus aureus was inhibited by a concentration of penicillin ranging from 0.12 to 0.25 units per ml.

Haemophilus influenzae isolated by direct culture of sputum or laryngeal swabs.

The results have been summarised in table 20.

Table 20

Days of examination	Resistance ratios: 1 & under	2	4	8 & over
1st	11			19
2nd		1		9
5th				2
10th	1			6

It can be seen that most of the strains of Haemophilus influenzae isolated had a high resistance ratio. There was also a group of organisms highly sensitive to penicillin. With the exception of one strain these were isolated on the first day. The strain isolated on the tenth day was obtained from a patient from whom there had been no previous isolation of Haemophilus influenzae. Five of these strains were inhibited by a concentration of 0.03 units per ml. and seven by a concentration of 0.06 units per ml. As all strains of Haemophilus influenzae isolated were unencapsulated, this degree of sensitivity is an unusual finding.

Haemophilus influenzae isolated by mouse inoculation.

Three strains were isolated by mouse inoculation. Two had a resistance ratio of eight and over and were cultured on the first and second day. One strain isolated on the first day had a ratio of under one. The inhibitory concentration of penicillin was 0.03 units per ml.

Sensitivity to tetracycline.

The standard sensitive Staphylococcus aureus was inhibited by a concentration of tetracycline which ranged from 2 to 4 mcg. per ml.

Haemophilus influenzae isolated by direct culture of sputum and laryngeal swabs.

The results have been summarised on table 21.

Table 21.

Days of examination.	Resistance ratios: 1 & under	2	4	8 & over
1st	21	7	1	1
2nd	7	1	1	1
5th	2	0	0	0
10th	7	0	0	0

It can be seen that most of the strains, thirty seven, had a resistance ratio of one or under. Eight organisms had a resistance ratio

of two, two a ratio of four, and two a ratio of eight. The organisms with the higher resistance ratios were isolated on the first and second days. Strains isolated on the fifth and tenth days had resistance ratios of one or under.

Haemophilus influenzae isolated by mouse inoculation.

Three strains were isolated, two on the first day and one on the second day. All had resistance ratios of one or under.

Sensitivity to streptomycin

The standard sensitive Staphylococcus aureus was inhibited by a concentration of streptomycin which ranged from 2 to 4 mcg per ml.

Haemophilus influenzae isolated by direct culture of sputum and laryngeal swabs.

The results have been summarised in table 22.

Table 22.

Days of examination.	Resistance ratios: 1 & under	2	4	8 & over
1st	26	1	2	1
2nd	9	0	0	1
5th	2	0	0	0
10th	5	0	0	2

Most of the strains, forty two had a resistance ratio of one or under. Four strains had a resistance ratio of eight and over. Although two of these were found on the tenth day the majority of organisms isolated on this day had a ratio of one or under.

Haemophilus influenzae insolated by mouse inoculation.

Three strains were isolated, two on the first day and one on the second day. All had resistance ratios of one or under.

Chloramphenicol

All strains isolated were sensitive with ratios of under one.

There were four additional strains of Haemophilus influenzae isolated from sputum which were not tested by the plate dilution method as the cultures died out before this was done. These strains were isolated on the fifth day and the disk sensitivities of all strains were - penicillin resistant, streptomycin sensitive, tetracycline sensitive and chloramphenical sensitive.

Klebsiella pneumoniae

Klebsiella pneumoniae isolated by direct culture of sputum and laryngeal swabs.

Sensitivity to penicillin

Ten strains were tested. All were resistant to penicillin. The inhibitory concentration for all strains was greater than 8 units per ml.

Tetracycline

The standard Staphylococcus aureus was inhibited by a concentration of tetracycline which ranged from 2 to 4 mcg. per ml.

The results have been summarised in table 23.

Table 23

Days of examination	Resistance ratios 1	2	4	8
1st		1	2	
2nd			1	
5th			2	1
10th			2	1

No marked variation in the sensitivity of the strains to tetracycline was observed. The majority had a ratio of four.

Streptomycin

The inhibitory concentration of the standard Staphylococcus aureus was from 2 to 4 mcg. per ml.

The results have been summarised on table 24.

Table 24.

Days of examination	Resistance ratios: 1	2	4	8
1st	3			
2nd	1			
5th	2			1
10th	3			

With one exception all the strains were sensitive to streptomycin. The resistant strain was inhibited by a concentration greater than 128 mcg. per ml. which was very high when compared with inhibitory concentrations of 1 and 2 mcg per ml. found in the sensitive strains.

Chloramphenicol

The standard sensitive Staphylococcus aureus was inhibited by 8 mcg. per ml.

The results have been summarised on table 25.

Table 25

Days of examination	Resistance ratios 1	2	4	8
1st	3			
2nd	1			
5th	1		1	1
10th	1	1		1

Most of the strains had a resistance ratio of under one or two. Of the strains with a higher resistance ratio, one was considered as definitely resistant, as the inhibitory concentration of chloramphenicol was greater than 128 mcg per ml. This strain was isolated on the tenth day.

Mouse inoculation

One strain was mouse virulent. It was isolated at the fifth day examination.

The inhibitory concentrations of the drugs were

Penicillin greater than 8 units per ml.

Tetracycline 16 mcg. per ml.

Streptomycin 4 mcg. per ml.

Chloramphenicol 8mcg. per ml.

Increase in resistance to antibiotics observed in organisms isolated on more than one occasion.

Increase in resistance to penicillin by pneumococci.

As the pneumococcus in an organism which is considered to be extremely sensitive to the action of penicillin, an unusual and unexpected finding in the present investigation was the presence of some strains which showed resistance to this antibiotic.

On blood agar, penicillin resistant pneumococci grew as round flat colonies with a raised outer edge, and alpha haemolysis was produced on heated blood agar. In broth the growth was agranular. Films of the organisms stained with India ink showed the presence of capsules. The presence of capsules was also demonstrated by type specific sera when these became available.

They were bile insoluble and optochin resistant. A few strains fermented inulin.

Organisms with these characteristics were inhibited by concentrations of penicillin ranging from 0.5 to 8 units per ml.

Penicillin resistance in pneumococci occurred as

(1) Primary resistance

(2) Acquired resistance

Primary resistance

This was regarded as an increased resistance to penicillin in pneumococci isolated at the start of the pneumonia where there was no evidence of previous treatment with the drug.

In sensitive strains of pneumococci the inhibitory concentration of penicillin was from 0.015 to 0.25 units per ml.

Pneumococci showing primary resistance have been divided into two groups.

1. Pneumococci isolated before mouse inoculation was performed and typing serum was available.
2. Pneumococci isolated when these tests were performed.

There were two patients in group 1 and four patients in group 2. Relevant details of these cases are appended.

Group 1

1. Male patient aged 82 years with a severe infection treated with penicillin and streptomycin. Pneumococci inhibited by a concentration of penicillin of 0.5 units per ml. were isolated

on the first day. On the second day pneumococci inhibited by a concentration of 1.0 units per ml. were isolated. The inhibitory concentration of streptomycin was 8 mcg. per ml. on the first day and 16 mcg. per ml. on the second day. Radiological clearing was normal and clinical response was satisfactory.

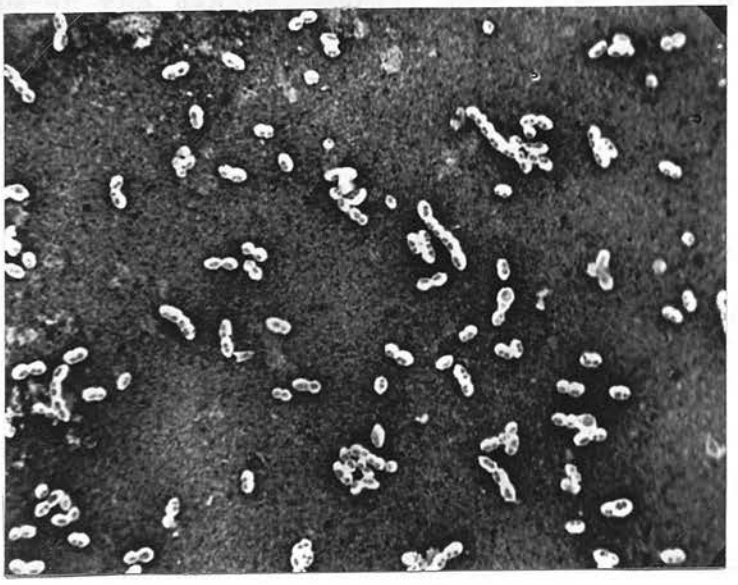
2. Male patient aged 58 years with a moderate infection treated with penicillin and streptomycin. Pneumococci inhibited by a concentration of penicillin of 4 units per ml. were isolated on the first day. The inhibitory concentration of streptomycin was 16 mcg. per ml. The only other specimen examined was on the tenth day and this was negative. Radiological clearing was considered to be slow. Clinical response was satisfactory.

Group 2.

There were four patients in this group.

1. Male patient aged 60 years with a severe infection. A type 3 mouse virulent pneumococcus inhibited by 4 units of penicillin per ml. was isolated on the first day. Blood culture also yielded a growth of type 3 pneumococci which were inhibited by 4 units of penicillin per ml. No further specimens were received as the patient died 12 hours after admission. Treatment was

Culture of type 3 pneumococcus isolated from blood of patient with severe lobar pneumonia. The organism was bile insoluble, optochin resistant and fermented inulin. The inhibitory concentration of penicillin was 4 units per ml. The patient died 12 hours after admission to hospital.



with antibiotics other than penicillin and streptomycin. The organism isolated by blood culture is illustrated on page293.

2. Female aged 30 years with a moderate illness treated with penicillin. A type 8 pneumococcus was isolated by mouse inoculation on the first day. It was not isolated by direct culture of the sputum. The organism was inhibited by 0.5 units per ml. of penicillin. Examination of the sputum on the second, fifth, and tenth days was negative. Radiological clearing was normal and the clinical response was satisfactory.

3. Female aged 46 years with a moderate infection treated with penicillin. A mouse virulent type 32 pneumococcus was isolated from the sputum on the first day. The inhibitory concentration of penicillin was 0.5 units per ml. No specimen was received on the second day. On the fifth day a type 32 pneumococcus inhibited by 1.0 units of penicillin per ml. but which was not mouse virulent was isolated. Radiological clearing was normal and the clinical response was satisfactory.

4. Female aged 33 years with a moderate illness treated with penicillin. A type 3 pneumococcus was isolated by mouse inoculation on the first day. It was not isolated by direct culture of the sputum. The inhibitory concentration of

penicillin was 4 units per ml. Bacteriological examinations of the sputum on the second, fifth, and tenth days were negative. Radiological clearing was considered slow and the clinical response satisfactory.

The number of cases is too small for the results to be of statistical significance. With this in mind the following observations were made.
(1) There seemed to be a relationship between a high inhibitory concentration of penicillin and a delayed radiological clearing
(2) Type 3 pneumococcal infections appeared to adversely affect the progress of the patient.
(a) One patient with this infection died and
(b) the other had a delayed radiological clearing.
(3) The inhibitory concentration of penicillin was higher when the infecting type was type 3, as compared with the level attained with the other two types (32 and 8) identified.

Acquired resistance

In all but one case this was regarded as an increased resistance to penicillin in strains of pneumococci isolated during later examinations when pneumococci isolated on the first day of the pneumonia were sensitive to the action of the drug. One case was placed in this group as there

was a recent history of infection with a penicill-sensitive pneumococcus which was treated with penicillin.

The cases have been divided into two groups.
1. Pneumococci isolated before mouse inoculation was performed and typing serum was available.
2. Pneumococci isolated when these tests were performed.

Group 1

The inhibitory concentrations of penicillin in units per ml. on the several days of examination have been tabulated below.
'0' signifies that no pneumococci were isolated on these days, '-' signifies that no specimen was received.

Patient	age	1st day	2nd day	5th day	10thday
1	66	.06	0	1	2
2	33	.015	0	0.5	0
3	71	.03	.06	0	2
4	59	.015	0	0	2
5	56	0	0.12	0	1
6	23	.012	0.12	0	8
7	61	.06	1.0	0	0
8	64	.12	1.0	0	0
9	75	.06	0.5	0	0
10	65	2	0	-	-

Further relevant details of these patients are appended.

Patient 1.

Female aged 66 years with a moderate infection treated with penicillin. The clinical response was satisfactory and the rate of radiological clearing was normal.

Patient 2.

Male aged 33 years with a severe infection treated with penicillin and streptomycin. The inhibitory concentration of streptomycin was 16 mcg. per ml. on the first day and 128 mcg. per ml. on the fifth day. The clinical response was satisfactory and the rate of radiological clearing was slow.

Patient 3.

Male aged 71 years with a moderate infection treated with penicillin. Clinical response was satisfactory and the rate of radiological clearing was slow.

Patient 4.

Male patient aged 59 years with a severe infection treated with pemicillin. The clinical response was satisfactory and the rate of radiological clearing was normal.

Patient 5

Male patient aged 56 years with a moderate infection treated with penicillin. Clinical response was satisfactory and the rate of radiological clearing was normal.

Patient 6

Female aged 23 years with a moderate infection treated with penicillin. Clinical response was satisfactory and the rate of radiological clearing was normal.

Patient 7

Male aged 61 years with a moderate infection treated with penicillin. Clinical response was satisfactory and the rate of radiological clearing was slow.

Patient 8

Male aged 64 years with a moderate infection treated with penicillin. Clinical response was satisfactory and the rate of radiological clearing was normal.

Patient 9

Male aged 75 years with a severe infection treated with penicillin. Clinical response was satisfactory and the rate of radiological clearing was normal.

Patient 10.

Male aged 65 years. Six weeks previously he was admitted to another hospital with pneumo-

coccal pneumonia which was treated with penicillin. On discharge after three weeks in hospital, the X ray examination was satisfactory. He was admitted to the City Hospital with dyspnoea, cyanosis, an elevated temperature (101°F) and E.S.R. of 36 m.m. per hour. The diagnosis then was severe broncho pneumonia and the treatment given was penicillin and streptomycin. He died 24 hours after admission. At autopsy there was histological evidence of a reticulum-cell sarcoma involving mainly the bone marrow. Primitive cells were observed in the blood vessels of the alveoli. There was also evidence of broncho pneumonia and chronic bronchitis.

When the first nine patients were considered, there was no apparent association between the age of the patients, the severity of the illness, the radiological clearing rate and the level of the inhibitory concentration of penicillin. However as serological typing was not undertaken it cannot be assumed that the organisms isolated in the later stages were the same strains as those isolated at the start of the illness.

In the 10th case there was evidence, assuming the inf ecting strain of pneumococcus was the same as that isolated previously, that resistance had

developed during the course of treatment. As this case is complicated by the presence of chronic bronchitis, a reticulum cell sarcoma and acute infection, it is difficult to assess which condition was primarily responsible for the suddenness of his death 24 hours after admission.

Group 2

There were four patients in this group.

The inhibitory concentrations of penicillin in units per ml. on the several days of examination have been tabulated below.

'0' signifies that no pneumococci were isolated on these days.

'-' signifies that no specimen was received.

Patient	Age	1st day	2nd day	5th day	10th day	Type
1	21	0.06	0.12	0	2	Not typed
2	59	0.25	2.0	0	0	"
3	49	0.25	0.12	0	1.0	"
4	56	0.03	0.5	0.5	0	3

Further relevant details of these patients are appended.

Patient 1

Male aged 21 years with a moderate infection treated with penicillin. Clinical response was

satisfactory and the rate of radiological clearing was normal. The organism isolated on the 10th day was not mouse virulent.

Patient 2.

Male aged 59 years with a moderate infection treated with penicillin. Clinical response was satisfactory and the rate of radiological clearing was normal. Both organisms were mouse virulent.

Patient 3.

Male aged 49 years with a moderate infection treated with penicillin. Clinical response was satisfactory and the rate of radiological clearing was slow. In addition to the three strains listed above, an extra sputum examination was done on the third day when a mouse virulent pneumococcus with an inhibitory concentration of 1 unit per ml. was isolated. All organisms isolated from this patient were mouse virulent.

Patient 4.

Male aged 56 years with a moderate infection treated with penicillin. Clinical response was satisfactory and the rate of radiological clearing was slow. All three strains were identified as type 3 pneumococci and all were mouse virulent.

All patients were under 60 years of age and suffered a moderate illness. Radiological clearing was slow in two cases but there did not appear to be an association between this and the age of the patient or the level of the inhibitory concentration of penicillin.

It was possible to type only one strain (type 3) therefore it is not known if the organisms isolated at later examinations were the same as those isolated at the start of the illness.

It can be seen that the radiological clearing in the case infected with the type 3 pneumococcus was delayed, a finding which was present in one case infected with this type where the resistance was primary.

Gram - positive diplococci which were penicillin resistant.

In addition to the pneumococci with an increased resistance to penicillin which were morphologically and colonially typical, there were observed in certain patients small Gram - positive diplococci which were resistant to penicillin and avirulent to mice. They were isolated from the sputum in the later stages of pneumococcal infections. The colonies were small with alpha haemolysis and had a raised outer edge.

In broth the culture was slow growing and agranular. They were bile insoluble, optochin insensitive, and usually fermented inulin.

As it was considered these organisms might be related to the pneumococcus, it was decided to explore the possibility of reversing them to capsulated pneumococci.

In the early stages of the trial, passage experiments using the intraperitoneal route had not been successful in restoring virulence to avirulent pneumococci. In view of this failure which may have been influenced by the use of 18 hour cultures, as these are frequently less mouse virulent than 6 hour cultures, (Cruickshank 1933), it was decided to employ subcutaneous inoculation. The methods used were those of Griffith (1928) who had been successful in reversing rough strains of pneumococci into smooth capsulated strains.

Material and methods.

The avirulent cultures referred to in these experiments were the small Gram-positive mouse avirulent acapsulated diplococci isolated from the sputum of certain patients with pneumococcal pneumonia on the 5th or 10th day of the illness.

The virulent cultures referred to were freshly isolated, virulent, type specific, cultures of pneumococci which were sensitive to penicillin. They were not obtained from the patients from whom the avirulent cultures were isolated.

Two groups of experiments were carried out.

In the first group the deposit of 50ml. of an avirulent culture was inoculated subcutaneously in a mouse. The site of inoculation was the root of the tail. A control was made by inoculating 0.5 ml. of the culture intraperitoneally in another animal.

In the second group of experiments the killed deposit of 50 ml. of a virulent pneumococcal culture was inoculated together with 0.5ml. of a live avirulent culture.

The virulent culture was killed by heating the deposit at 60°C for 30 minutes.

Controls were made as follows.

1. The killed culture was tested for sterility by inoculating on blood agar.
2. The killed culture only was inoculated subcutaneously.
3. The avirulent culture was inoculated alone both subcutaneously and intraperitoneally.

The site of inoculation was the root of the tail.

These experiments and the results obtained have been summarised in tables 26 and 27 and are given in detail below.

In the group where the inoculum was the deposit of 50ml. of avirulent penicillin resistant culture, seven experiments were performed. In one of these a type specific penicillin resistant pneumococcus was recovered. The details of the experiments are given below, and are summarised on table 26.

Experiment 1.

The patient in this experiment was a female aged 66 years with a severe pneumococcal pneumonia where a type 3 pneumococcus sensitive to penicillin (minimum inhibitory concentration of penicillin 0.03 units per ml.), was isolated on the first day. The avirulent culture was isolated on the fifth day from the sputum and was inhibited by a concentration of penicillin of 8 units per ml.

Three days after inoculation with the avirulent culture, a pustular lesion appeared at the site of inoculation. The pus was cultured in

serum broth when Gram positive diplococci which were not typable were seen. 0.5 ml. of this culture inoculated intraperitoneally in a mouse caused death in 18 hours. Morphologically typical pneumococci were isolated from the heart blood, pleural fluid and peritoneal fluid. These organisms were bile insoluble, optochin resistant and fermented inulin. The growth in serum broth was cloudy and agranular. There was capsule swelling with type 3 serum. The inhibitory concentration of penicillin was 8 units per ml.

Details of the remaining experiments are given below.

Experiment 2.

The avirulent culture was inhibited by a concentration of 8 units per ml. It was isolated from the sputum of the patient described in experiment 1, on the tenth day of the pneumonia.

Three days after inoculation with the avirulent culture, a pustular lesion appeared at the site of inoculation. Gram-positive diplococci and Gram-negative bacilli were seen in a film of the pus. Culture of the pus yielded a growth

of Proteus. It was considered that this was possibly the result of contamination from the site of inoculation.

Experiment 3

The avirulent culture was inhibited by a concentration of penicillin of 8 units per ml. It was isolated from the sputum on the tenth day from a patient with pneumococcal pneumonia from whom a type 9 pneumococcus sensitive to penicillin (minimum inhibitory concentration of penicillin of 0.03 units per ml) was isolated on the first day of the pneumonia. No lesion was observed at the site of inoculation.

Experiment 4

The avirulent culture was inhibited by a concentration of penicillin of 4 units per ml. It was isolated from the sputum on the fifth day from a patient with pneumococcal pneumonia from whom a type 4 pneumococcus sensitive to penicillin (minimum inhibitory concentration of penicillin was 0.03 units per ml) was isolated on the first day of the pneumonia. No lesion was observed at the site of inoculation.

Experiment 5

The avirulent culture was inhibited by a concentration of penicillin of 4 units per ml. and was isolated from the sputum on the fifth day from a patient with pneumococcal pneumonia, from whom a type 32 pneumococcus inhibited by a concentration of penicillin of 0.015 units per ml. was isolated on the first day. Three days after inoculation, a pustular lesion appeared at the site of inoculation. The pus was cultured in serum broth for 18 hours when Gram-positive diplococci which were not typable were seen. 0.5ml. of this culture inoculated intraperitoneally in a mouse was found to be avirulent. No growth was obtained on culture of the peritoneal washings and heart blood. Subcutaneous inoculation of the deposit of 50ml. of this culture in a mouse did not produce a lesion.

Experiment 6

The avirulent culture was inhibited by a concentration of penicillin of 4 units per ml. It was isolated on the fifth day from the sputum of a patient with pneumococcal pneumonia from whom a type 35 pneumococci were isolated on the first day of the pneumonia. These were penicillin sensitive, being inhibited by a concentration

of 0.03 units per ml. No lesion was observed at the site of inoculation.

Experiment 7

The avirulent culture was inhibited by a concentration of 4 units per ml. It was isolated from the sputum on the fifth day from a patient with a pneumococcal pneumonia from whom a type 20 pneumococcus sensitive to penicillin (minimum inhibitory concentration of penicillin was 0.015 units per ml) was isolated on the first day. No lesion was observed at the site of inoculation.

In the second group of experiments the inoculum was the deposit of 50ml. of killed virulent culture combined with 0.5ml. of a live avirulent culture.

The results of the experiments have been summarised on table 27. The details are given below.

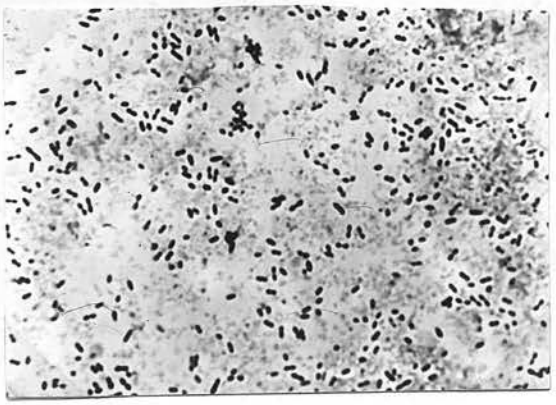
Experiment 8

The avirulent culture was isolated from the sputum of a female patient aged 66 years on the fifth day of the illness. The organism was inhibited by a concentration of penicillin of 8 units per ml. A type 3 penicillin sensitive pneumococcus (minimum inhibitory concentration of penicillin was 0.03 units per ml.) was isolated on the first day of the pneumonia.

The virulent culture was a type 3 pneumococcus isolated from another patient.

Four days after inoculation a pustular lesion appeared at the site of inoculation. The pus was cultured in serum broth when Gram-positive diplococci which were not typable were observed. 0.5ml. of this culture inoculated intraperitoneally in a mouse caused death in 18 hours. Morphologically typical pneumococci were

Culture of Gram positive diplococcus isolated from the sputum of a patient with pneumonia on the tenth day of the illness. The organism was bile insoluble, optochin insensitive, acapsular, fermented inulin and was mouse avirulent. The inhibitory concentration of penicillin was 8 units per ml.



isolated from the heart blood. They were bile insoluble, optochin resistant and fermented inulin. In broth the growth was cloudy and agranular. The inhibitory concentration of penicillin was 8 units per ml. There was capsule swelling with type 3 serum. The avirulent organism isolated from this patient is illustrated on page 311.

Experiment 9

The avirulent culture was isolated from the sputum of a female patient aged 75 years on the tenth day of the illness. The organism was inhibited by a concentration of 8 units per ml. A type 23 pneumococcus sensitive to penicillin (minimum inhibitory concentration of penicillin was 0.03 units per ml.) was isolated from the blood on the first day of the illness. In this case no sputum specimen was available on this day and culture of a laryngeal swab was negative.

The virulent culture was a type 23 pneumococcus isolated from another patient.

Four days after inoculation, a pustular lesion appeared at the site of inoculation. The pus was cultured in serum broth when Gram-positive diplococci which were not typable were observed.

0.5ml of this culture when inoculated intraperitoneally in a mouse caused death in 18 hours. Morphologically typical pneumococci were isolated from the heart blood. They were bile insoluble, optochin insensitive, and fermented inulin. In broth the growth was cloudy and agranular. The inhibitory concentration of penicillin was 8 units per ml. There was capsule swelling with type 23 serum.

Experiment 10

The avirulent culture was isolated from the sputum of a female patient aged 50 years, on the tenth day of the pneumonia. The organism was inhibited by a concentration of penicillin of 8 units per ml. A type 23 pneumococcus inhibited by a concentration of 0.03 units per ml. was isolated from the sputum on the first day of the pneumonia.

The virulent culture was a type 8 pneumococcus isolated from another patient.

Four days after inoculation, a pustular lesion appeared at the site of inoculation. The pus was cultured in serum broth for 18 hours when

Gram-positive diplococci which were not typable were seen. 0.5ml. of this culture inoculated intraperitoneally in a mouse was avirulent to the animal and the mouse was killed after three days. Culture of the peritoneal washings and heart blood was sterile. 0.5ml. of the peritoneal washings was inoculated intraperitoneally in another mouse and proved to be avirulent. The peritoneal washings were inoculated in another animal with the same negative result. This procedure was repeated with four further mice. No growth was obtained from the peritoneal washings or heart blood of these animals.

Experiment 11.

The avirulent culture was isolated from the sputum of a male patient aged 36 years on the tenth day of the pneumonia. The organism was inhibited by 8 units per ml. of penicillin. A type 4 pneumococcus inhibited by 0.03 units per ml. of penicillin was isolated from the sputum on the first day of the illness.

The virulent culture was a type 4 pneumococcus isolated from another patient.

Three days after inoculation a pustular lesion appeared at the site of inoculation.

The pus was cultured in serum broth for 18 hours when Gram-positive diplococci which were not typable were seen. 0.5ml. of this culture inoculated intraperitoneally in a mouse was avirulent to the animal and the mouse was killed after three days. Culture of the peritoneal washings and heart blood was sterile. 0.5ml. of the peritoneal washings was inoculated intraperitoneally in another mouse and proved to be avirulent and sterile. The peritoneal washings were inoculated in another mouse with the same negative results. This procedure was repeated with four further mice. No growth was obtained from the peritoneal washings or heart blood of these animals.

Experiment 12.

The avirulent culture was isolated from the sputum of a male aged 82 years on the tenth day of the illness. The organism was inhibited by 8 units per ml. of penicillin. A type 9 pneumococcus penicillin sensitive (minimum inhibitory concentration of penicillin was 0.03 units per ml.) was isolated on the first day of the pneumonia.

The virulent culture was a type 20 pneumococcus isolated from another patient.

No lesion was observed at the site of inoculation.

Experiment 13

The avirulent culture was isolated from the sputum of a male patient aged 55 years on the tenth day of the pneumonia. The organism was inhibited by 8 units per ml. of penicillin. A type 1 penicillin sensitive pneumococcus was isolated on the first day of the illness. The inhibitory concentration of penicillin was 0.03 units per ml.

The virulent culture was a type 21 pneumococcus isolated from another patient.

Three days after inoculation a pustular lesion appeared at the site of inoculation. The pus was cultured in serum broth for 18 hours when Gram-positive diplococci which were not typable were seen. 0.5ml. of this culture inoculated intraperitoneally in a mouse was avirulent to the animal and the mouse was killed after three days. Culture of the peritoneal washings and heart blood was sterile. 0.5ml. of the peritoneal

washings was inoculated intraperitoneally in another mouse and proved to be avirulent and sterile. The peritoneal washings were inoculated in another mouse with the same negative results. This procedure was repeated with four further mice. No growth was obtained from the peritoneal washings or heart blood of these animals.

Experiment 14.

The avirulent culture was isolated from the sputum of a male patient aged 60 years on the tenth day of the pneumonia. The organism was inhibited by 8 units of penicillin per ml. A type 21 pneumococcus penicillin sensitive (minimum inhibitory concentration of penicillin was 0.03 units per ml) was isolated on the first day of the illness.

The virulent culture was a type 8 pneumococcus isolated from aother patient.

No lesion was observed at the site of inoculation.

Two of the experiments (8 and 9) resulted in the production of a type specific penicillin resistant pneumococcus.

In these experiments the pneumococcal type isolated at the start of the pneumonia, was the same as that used as the transforming strain, namely type 3 in experiment 8 and type 23 in experiment 9.

It was considered that the pneumococci which had caused the pneumonias were of a virulent nature. The virulence of type 3 is well known. Although type 23 is a higher type, in this patient it was cultured from the blood which is an indication of its invasive nature.

In three experiments (10,11 and 13) Gram-positive diplococci were isolated from the mouse but were avirulent on further inoculation. These organisms were cultured for 18 hours. It is possible a more virulent culture would have been obtained if the incubation time had been shorter. Cruickshank (1933) considered that mouse virulence was greatest in young cultures of pneumococci and recommended the use of six-hour cultures.

Subcutaneous experiments

Table 26.

Inoculation of deposit of 50 ml. of avirulent penicillin resistant culture.

Expt.	Source of avirulent culture	Concentration of penicillin which inhibited growth of avirulent culture	Pneumococcal type isolated at start of pneumonia	Result of mouse inoculation
1	Sputum on 5th day of pneumonia	8 units per ml.	3	Type 3 pneumococcus inhibited by 8 units per ml. of penicillin.
2	Sputum on 10th day of pneumonia	8 units per ml.	3	Culture was contaminated
3	Sputum on 10th day of pneumonia	8 units per ml.	9	Negative
4	Sputum on 5th day of pneumonia	4 units per ml.	4	Negative
5	Sputum on 5th day of pneumonia	4 units per ml.	32	Gram positive diplococci isolated from subcutaneous lesion. No growth obtained on further inoculation.
6	Sputum on 5th day of pneumonia	4 units per ml.	35	Negative
7	Sputum on 5th day of pneumonia	4 units per ml.	20	Negative

TABLE 27

Inoculation of deposit of 50 ml. of killed virulent culture with 0.5 ml. of live avirulent culture

Expt.	Source of avirulent culture	Concentration of penicillin which inhibited growth of avirulent culture	Pneumococcal type isolated at start of pneumonia.	Type of virulent culture	Result of mouse inoculation
8	Sputum on 5th day of pneumonia	8 units per ml.	3	3	Type 3 pneumococcus inhibited by 8 units per ml. of penicillin isolated.
9	Sputum on 10th day of pneumonia	8 units per ml.	23	23	Type 23 pneumococcus inhibited by 8 units per ml. of penicillin isolated.
10	Sputum on 10th day of pneumonia	8 units per ml.	23	8	Gram-positive diplococci isolated from subcutaneous lesion. No enhancement of virulence after passage.
11	Sputum on 10th day of pneumonia.	8 units per ml.	4	4	Gram-positive diplococci isolated from subcutaneous lesion. No enhancement of virulence after passage.
12	Sputum on 10th day of pneumonia.	8 units per ml.	9	20	Negative

TABLE 27 (cont.)

Expt.	Source of avirulent culture	Concentration of penicillin which inhibited growth of avirulent culture.	Pneumococcal type isolated at start of pneumonia.	Type of virulent culture	Results of mouse inoculation
13	Sputum on 10th day of pneumonia.	8 units per ml.	1	21	Gram-positive diplococci isolated from subcutaneous lesion. No enhancement of virulence after passage.
14	Sputum on 10th day of pneumonia.	8 units per ml.	21	8	Negative.

Cases from which Gram-positive penicillin resistant diplococci were isolated.

The cases have been described according to the type of pneumococcus isolated at the start of the pneumonia and the experiments related to each patient are given.

1. Type 1

Male aged 55 years with a moderate infection due to a type 1 pneumococcus. Gram-positive penicillin resistant diplococci isolated on the tenth day. The clinical response and radiological clearing were satisfactory. (Experiment 13)

2. Type 3

Female aged 66 years with a severe infection due to a type 3 pneumococcus. Gram-positive penicillin resistant diplococci were isolated on the fifth and tenth days. Radiological clearing was slow and the patient died after 21 days. (Experiments 1, 2 and 8).

3. Type 4

Male aged 47 years with a severe infection due to a type 4 pneumococcus. Gram-positive penicillin resistant diplococci isolated on the fifth and tenth days. The clinical response and radiological clearing were satisfactory. (Experiments 4 and 11).

4. Type 9

Male aged 84 years with a moderate illness due to a type 9 pneumococcus. Gram-positive penicillin resistant diplococci isolated on the tenth day. The clinical response and radiological clearing were satisfactory. (Experiments 3 and 12).

5. Type 20

Male aged 75 years with a moderate illness due to type 20 pneumococcus. Gram-positive penicillin resistant diplococci isolated on the fifth day. Clinical response was satisfactory but radiological clearing was considered prolonged. (Experiment 7).

6. Type 21

Male aged 60 years with a moderate illness due to a type 21 pneumococcus. Gram-positive penicillin resistant diplococci isolated on the tenth day. Clinical response and radiological clearing was satisfactory (Experiment 14).

7. Type 23

Female aged 75 years with a moderate illness due to a type 23 pneumococcus. Gram-positive penicillin resistant diplococci isolated on the

10th day. Clinical response was satisfactory but radiological clearing was considered prolonged. (Experiment 9).

8. Type 23

Female aged 50 years with a moderate illness due to a type 23 pneumococcus. Gram-positive penicillin resistant diplococci isolated on the tenth day. Clinical response and radiologcial clearing were satisfactory (Experiment 10).

9. Type 32

Female aged 46 years with a moderate infection due to a type 32 pneumococcus. Gram-positive penicillin resistant diplococci isolated on the fifth day. Clinical response and radiological clearing were satisfactory (Experiment 5).

10. Type 35

Male aged 62 years with a severe illness due to a type 35 pneumococcus. Gram-positive penicillin resistant diplococci isolated on the fifth day. Clinical response and radiological clearing were satisfactory. (Experiment 6).

Ten patients were in this study and all of these but one were over 50 years of age. This

is probably not of significance as most of the pneumococcal types which had caused the infections were of the higher types and it had been observed that these were found more frequently in older patients in this investigation.

In the majority of patients the appearance of Gram-positive diplococci in the later stages of pneumonia did not have an adverse effect on either the clinical response or the rate of radiological clearing.

It is of note that in two of the three cases where radiological clearing was slow, reversion of the avirulent culture to a type specific penicillin resistant pneumococcus took place.

From one of these cases a type 23 pneumococcus was isolated at the start of the illness. In this case the clinical response was satisfactory. The second patient, who was infected by a type 3 pneumococcus, died 21 days after admission to hospital.

The finding in all penicillin resistant organism whether mouse virulent or not of bile insolubility, optochin insensitivity and sometimes failure of inulin fermentation, prompted an investigation into the *in vitro* effect of penicillin on pneumococci.

This was done by growing the pneumococci in increasing concentrations of penicillin and observing the reactions of the organisms to the above tests during growth in the various concentrations of the drug.

Culture of pneumococci in increasing concentrations of penicillin.

Material and methods

The medium employed was 10% serum broth and the required amount of penicillin was added.

Only pneumococci which had the following characters were used.

(1) Bile solubility, (2) optochin sensitivity (3) ability to ferment inulin (4) type specificity (5) mouse virulence.

At the start of the investigation the minimum inhibitory concentration of penicillin for the strain was determined.

The concentrations of penicillin which were used were -
.025, 0.05, 0.1, 0.2, 0.4, 0.8, 1.0, 1.5, 2.0, 2.5, and 3.0 units per ml.

Two loopfuls of the broth culture containing the highest concentration of penicillin which would permit growth were inoculated into 10% serum broth containing the next higher concentration of penicillin until no further advance in penicillin could be obtained.

The penicillin serum broth culture was incubated for 18 hours when if growth had taken place it was subcultured on a blood agar plate and heated blood agar plate in order that the morphology and colonial character of the pneumococcus could be studied.

The reactions of the organism to bile, optochin, and inulin were checked after each exposure to the drug.

Mouse virulence was checked at intervals and type specificity at each isolation.

The types of pneumococci investigated were

Type 3	3 strains
" 4	1 strain
" 5	2 strains
" 8	2 strains
" 10	3 strains
" 11	4 strains
" 13	2 strains
" 14	3 strains
" 17	1 strain
" 21	1 strain
" 23	2 strains
" 32	4 strains
" 34	2 strains

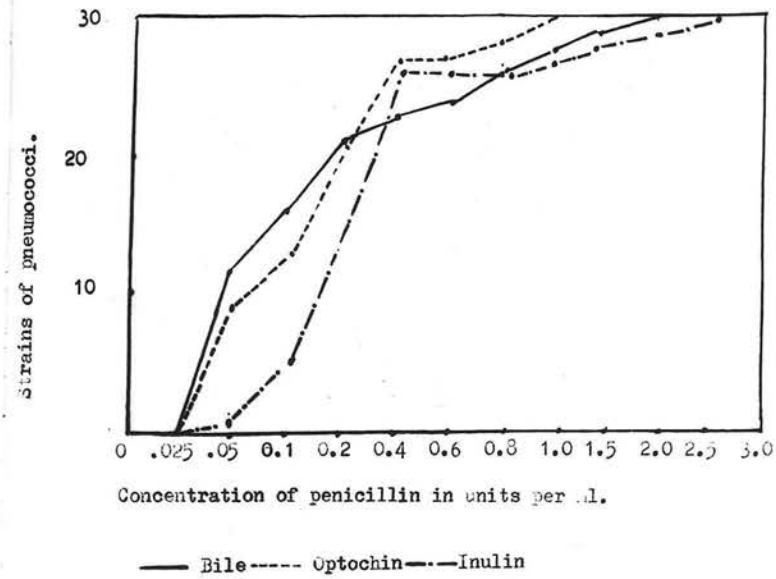
It was found that as pneumococci were grown in increasing concentrations of penicillin, the properties of bile solubility, optochin sensitivity and inulin fermentation were lost.

The results of this experiment are seen on Figure 2 on page 329.

It can be seen there was a variation in the concentrations of penicillin causing the loss of these properties. Nine of the thirty strains became optochin resistant when grown in 0.05 units

Figure 2

The in vitro effect of penicillin on thirty strains of pneumococci showing the number of strains which lost the properties of sensitivity to optochin, solubility in bile, and fermentation of inulin when grown in increasing concentrations of penicillin.



per ml. while two out of thirty retained this property until grown in 1.0 units per ml. In a concentration of 0.05 units per ml. twelve of the thirty became bile insoluble. One strain retained this property until grown in 2.0 units per ml. In the lower concentrations of penicillin a larger number of organisms retained the property of inulin fermentation as compared with bile solubility and optochin sensitivity. In a concentration of 0.1 units per ml., five of the thirty strains did not ferment inulin. At this concentration, thirteen strains were optochin insensitive and sixteen bile insoluble.

Mouse virulence was tested at intervals but was found to be lost when the organisms were growing in low concentrations of penicillin. The cultures were subcultured frequently.

Type specificity was lost by ten strains when grown in 0.8 units per ml. and by twenty strains when grown in 1.0 units per ml.

Films made from the cultures showed that the organisms became smaller when grown in

increasing concentration of penicillin.

It was also observed that a few of the cultures became Gram-negative either completely or in part.

Growth in serum broth was agranular.

It appeared that all the pneumococcal types studied, acquired resistance to penicillin at a comparatively equal rate.

On blood agar the colonies became progressively smaller as the concentration of penicillin increased. This diminution in size of the colonies was also observed on heated blood agar where alpha ahemolysis was present.

It was found that the same level of resistance to penicillin was retained after 60 subcultures in a penicillin free medium.

From the results of this experiment it appeared there was a resemblance between pneumococci grown in progressively increasing concentrations of penicillin, and the Gram-positive diplococci isolated during the later stages of the pneumonia, from the sputum of patients with pneumococcal pneumonia treated with penicillin.

Both were penicillin resistant, small Gram-negative acapsulated, mouse avirulent organisms producing small colonies on solid medium and an agranular growth in broth. In both the properties of bile solubility and optochin fermentation were lost. It appeared that inulin fermentation was lost more readily by pneumococci grown in penicillin than by the Gram-positive diplococci isolated from the sputum.

Increase in resistance to tetracycline and streptomycin in strains of pneumococci isolated at more than one examination.

Tetracycline

In two cases where pneumococci were isolated on the first and second days, the inhibitory concentration on the first day was 4 mcg. per ml and 16 mcg. per ml. on the second day. A type 3 pneumococcus was isolated on both days in one case.

In four cases where pneumococci were isolated on the first and tenth days, the inhibitory concentration on the first day was 4mcg. per ml. and 16 mcg. per ml. on the tenth day. A type 3 pneumococcus was isolated on both days from one case.

Streptomycin

In two cases where pneumococci were isolated on the first and tenth days, the inhibitory concentration on the first day was 16 mcg. per ml, and 128 mcg. per ml. on the tenth day.

In one case where pneumococci were isolated on the first and fifth days, the inhibitory concentration on the first day was 1mcg. per ml. and 8 mcg. per ml. on the fifth day. In one case where pneumococci were isolated on the first and second days, the inhibitory concentration on the first day was 8mcg. per ml. and 16 mcg. per ml. on the second day.

In one case where pneumococci were isolated on the first and second days, the inhibitory concentration on the first day was 2 mcg. per ml. and 8 mcg. per ml. on the second day. Type 8 pneumococci were isolated on both days of examination.

No increase in resistance to chloramphenicol was observed in strains of pneumococci isolated at more than one examination.

Increase in resistance by Staphylococcus aureus to penicillin, streptomycin, and tetracycline

As phage typing was not done in this investigation, it cannot be definitely established that the strains isolated at the end of the pneumonia were the same as those found at the start of the illness.

Penicillin

Two strains were isolated where the inhibitory concentration rose from 0.12 units per ml. on the first day to 8 units per ml. on the second day.

One strain was isolated where the inhibitory concentration rose from 0.5 units per ml. on the first day to 16 units per ml. on the second day.

Streptomycin

Two strains were isolated where the inhibitory concentration rose from 4mcg. per ml., on the first day to 64 mcg. per ml. on the fifth day. One strain was isolated where the inhibitory concentration rose from 4mcg. per ml. on the first day to 128 mcg. per ml. on the fifth day.

Tetracycline.

One strain was isolated where the inhibitory concentration rose from 2mcg. per ml. on the second day to 16mcg. per ml. on the tenth day.

Chloramphenicol

No increase in resistance was observed with this drug.

Increase in resistance to penicillin in strains of Haemophilus influenzae isolated at more than one examination.

In two cases where Haemophilus influenzae was isolated on the first and second days, the inhibitory concentration on the first day was 0.06 units per ml. and 8 units per ml. on the second day.

In one case where Haemophilus influenzae was isolated on the first and fifth days, the inhibitory concentration on the first day was 0.03 units per ml. and 8mcg. per ml. on the fifth day.

As all strains of Haemophilus influenzae were unencapsulated, it cannot be assumed that these cases demonstrated an increase in resistance to penicillin.

No change in the sensitivity to streptomycin, tetracycline and chloramphenicol was observed in strains of Haemophilus influenzae isolated at more than one examination.

Bacteriological findings in fatal cases.

Brief histories of the fatal cases are appended.

1. Male patient aged 79 years with a left upper lobe pneumonia. On the first day a type 3 pneumococcus was isolated from the blood and sputum. A growth of Staphylococcus aureus and Klebsiella pneumoniae was also cultured from the sputum on the first day. Pneumococci and Klebsiella pneumonia were isolated on the second day and Staphylococcus aureus on the fifth day and tenth day. The patient died 19 days after admission.

2. Female patient aged 66 years with a left upper lobe pneumonia. On the first day a type 3 pneumococcus was isolated from the blood and sputum. On the fifth and tenth days penicillin resistant Gram-positive diplococci were isolated from the sputum. The organisms isolated on the fifth day were by mouse passage reversed to penicillin resistant type 3 pneumococci. Patient died 21 days after admission.

3. Male patient aged 65 years. A pneumococcus which was not typed was isolated on the first day.

This organism was inhibited by a concentration of penicillin of 2 units per ml. i.e. it was penicillin resistant. Six weeks previously this patient had received treatment for a pneumococcal pneumonia which appeared to respond to this therapy. He was admitted with an apparent relapse and died 24 hours after admission. Severe bronchopneumonia was diagnosed on clinical and post mortem examination. Histological examination showed the presence of a reticulum cell sarcoma involving mainly the bone marrow. Primitive cells were seen in the blood vessels of the alveoli. In addition this patient suffered from severe chronic bronchitis.

4. Male patient aged 60 years. On the first day a mouse virulent type 3 pneumococcus was isolated from the blood and sputum. This organism was inhibited by a concentration of penicillin of 4 units per ml. This patient was gravely ill on admission and died after 12 hours. There was consolidation of the left upper lobe and bronchopneumonic areas in both lower lobes.

5. Male patient aged 82 years, with left sided segmental pneumonia. A type 14 pneumococcus was isolated from the blood. Only laryngeal swabs were available for examination. Staphylococcus

aureus was isolated on first, second, fifth and tenth day examinations. All strains were sensitive to penicillin with an inhibitory concentration of 0.06 units per ml. and treatment was with penicillin. He was very ill on admission and there was no response to treatment. Death occurred after 13 days.

6. Female aged 17 years. On admission this patient was gravely ill with an extensive right sided lobar pneumonia. She had been quite well until the previous evening. No sputum was available and capsulated diplococci morphologically resembling pneumococci were seen in the film of the laryngeal swab. There were also Gram negative bacilli morphologically resembling Haemophilus influenzae. Culture of the swab was not satisfactory and pathogenic bacteria were not isolated by this method. A specimen of the blood was not cultured. The patient also had a pleural effusion which was sterile on culture, and mouse inoculation was negative. She died 6 hours after admission.

The patient was mentally backward but not a mongol. It is of interest that she had a 'cold' three weeks previously.

7. Male aged 70 years with lobular pneumonia. On the first day a growth of type 3 pneumococci and Haemophilus influenzae was obtained from the sputum. Although these organisms disappeared from the sputum the clinical response of the patient was not satisfactory. On the tenth day and on subsequent examinations until death, Ps pyocyanea was isolated from specimens obtained by tracheostomy. He died 18 days after admission. He also suffered from chronic bronchitis.

8. Female aged 88 years with lobular pneumonia. On the first day a type 6 pneumococcus was isolated and there was also a growth of Haemophilus influenzae at this examination. With penicillin therapy the chest infection improved but the patient developed diarrhoea after four weeks, her condition deteriorated, and death occurred eight weeks after admission. Post mortem examination showed a wide-spread diverticulitis with a perforation and peritonitis. Two weeks before the onset of pneumonia the patient had an upper respiratory tract infection.

9. Male aged 71 years with lobular pneumonia and a history of chronic bronchitis. On the first day a profuse growth of Staphylococcus

aureus sensitive to penicillin, streptomycin, tetracycline and erythromycin was isolated. The infection responded to treatment and he was discharged from hospital after eight weeks. During his stay in hospital he developed congestive cardiac failure which was controlled with digitalis. However this patient died at home in the month following discharge from hospital.

10. Male aged 63 years admitted with congestive failure and lobular pneumonia. A profuse growth of Staphylococcus aureus resistant to penicillin, streptomycin, and tetracycline was isolated on the first day and second day. The infection was controlled with crystalline penicillin and methicillin but the patient died of congestive cardiac failure 18 days after admission. This patient suffered from chronic bronchitis.

11. Male aged 61 years with lobular pneumonia. This patient had a long history of chronic bronchitis. He had received antibiotic treatment before admission and no pathogenic organisms were isolated from the one sputum specimen examined. The organisms found in this specimen were N. catarrhalis and Strept. viridans

With one exception the patients in this group were over 60 years of age. In 7 cases pneumococci were isolated on culture and were present in the Gram film only in one case.

Infection was most frequent with type 3 pneumococci. Of the six pneumococcal types found, four were identified as type 3.

Pneumococci were cultured from the blood of four patients. Three strains were identified as type 3 and one as type 14.

Three strains of pneumococci showed increased resistance to penicillin; in one strain the resistance was primary in type, and in the other two there appeared to be acquired resistance.

Staphylococcus aureus was isolated in four cases. In two cases it was the cause of pneumonia in patients with severe chronic bronchitis. In the remaining cases it was an added complication of pneumococcal infections in elderly patients.

DISCUSSION

This investigation of pneumonia was conducted on completely unselected cases of the disease. Interesting information has therefore emerged on the type of case which is of sufficient severity to require treatment in hospital. In this connection the points which are discussed include the nature of the infecting agents responsible for the pneumonias and the age groups which were affected. Much of the work in this study was concerned with determining the sensitivities of the pathogenic organisms to the antibiotics commonly used in the treatment of pneumonia. The results of these findings are considered with particular regard to the appearance of resistance to the drugs studied. An unexpected finding was the isolation of some strains of pneumococci which showed an increased resistance to penicillin, and these are considered with regard to their morphological and colonial appearance, their biochemical reactions and the nature of the illness caused by them. It was apparent from the bacteriological findings in the investigation, that the isolation rate of pathogenic organisms could be increased by the use of certain selective techniques. Some of these were employed from the start, and others

were introduced during the course of the study. There is a discussion on the value and necessity of the employment of such methods for a full understanding of the bacteriological aetiology of pneumonia. Finally there is a review of the cases in the investigation which had a fatal outcome.

The organisms isolated

The predominance of the pneumococcus in this investigation indicates the importance of this organism as the infecting agent in pneumonia. This is agreement with Austrian (1957, 1959) who considered that despite statements to the contrary, the pneumococcus remained the most important cause of bacterial pneumonia in the temperate zone. Other bacteriological investigations of pneumonia have shown that of the pathogenic bacteria isolated, the pneumococcus was responsible for the largest number of infections. In the report of the Antibiotics Clinical Trials Committee of the Medical Research Council (1951) on the treatment of pneumonia, where 267 cases from four hospitals were studied, it was shown that pneumococci were concerned in 196 cases. Grist and collaborators (1952) isolated pneumococci from 84 of 129 cases.

The number of cases due to pneumococcal infection in the study by Eadie and others (1951) was 63 out of 79. Stuart Harris (1953) investigated four series of cases with pneumonia and found the percentage of pneumococci isolated ranged from 61% to 81%. In the above studies details of the nature of the pneumonic infection are not given. Pneumococci were isolated from 26 cases with consolidation, and from 3 with lobular pneumonia in the study by Crofton and colleagues (1951). Israel and co workers (1948) found an isolation rate of pneumococci of over 90% in cases oflobar pneumonia. In the investigation by Humphrey and others (1948) of pneumonias with evidence of consolidation, pneumococci were isolated from 278 cases out of 351 studied.

Of particular interest in the present study was the tendency of the pneumococcus to infect older patients. This was seen in the male patients where 73% of pneumonias due to infection with the pneumococcus either alone or together with Haemophilus influenzae, occurred in patients of 50 years and over. When all the male patients in the investigation in this age group were considered, it was seen that a pneumococcal infection was present in 50% of cases Slightly more than half the pneumococcal

infections in females were in patients of 50 years and over, and these infections were responsible for about one third of the pneumonias in this age group. It is not considered however that a comparison of the male and female cases can be made as the latter were fewer in number and were restricted by the smaller number of beds available for female cases.

It is possible that the number of pneumococcal infections in the present study is an underestimate, as it is probable that some cases were not diagnosed bacteriologically because previous antibiotic treatment had masked the infecting agent.

It would appear from this investigation that in hospital practice, pneumococcal infections occur predominantly in older patients and are responsible for a large proportion of pneumonias found in this group of people.

The practice of typing pneumococci has now become relatively uncommon; it would seem to be of interest to identify the pneumococcal types which are now responsible for infections. Not all the pneumococcal types in the present study were identified, but interesting information was obtained from the small investigation which was undertaken.

It was considered that the high proportion

of type 3 pneumococci which were identified was of particular significance as four of the eleven fatal cases in the investigation were infected with this type. As eight of the eleven strains identified as type 3 pneumococci were isolated from patients over fifty years of age, it would appear that the tendency of this type to infect older people, commented on by Heffron (1939) is still present. It would seem that the high percentage (31%) of type 3 strains isolated, was related to the predominance of older patients in this investigation. Thomson and others (1951) and Humphrey and colleagues (1948) found a high incidence of type 3 pneumococcal infections in older patients.

In other studies where pneumococcal types were identified, there is not similar complete information on the age groups affected, and it is difficult to compare the findings of such investigations with the present series. A predominance of type 1 infections has been reported by certain investigators (Humphrey and colleagues, 1948; Israel and others, 1948; Austrian and Winston, 1956; Thomson and associates, 1951; Van Metre, 1954). It appeared from the information given by Humphrey and colleagues (1948) that there was a fairly wide distribution of patients of all ages although

there was an age limit of 70 years.

In an extensive study of epidemic pneumococcal infections in young adults by Hodges and MacLeod (1946), the predominant type was type 2 which is associated with infection in this age group. This was the predominant type in the investigations by Anderson and Ferguson (1945), Anderson and Landsman (1947) Eadie and colleagues (1951) and Grist and associated (1952). The last four studies were undertaken in Glasgow where there would appear to be a predominance of type 2 pneumococci.

In a study of the prevalence of pneumococcal types, Austrian (1959) isolated over a five year period 1,354 strains of pneumococci, 12% of which were identified as type 3 organisms. This was the most common type found in this series, and in this author's opinion was due to the ease with which type 3 pneumococci are cultured from sputum, isolated by mouse inoculation, and their frequent occurrence in the carrier state. There is however no information on the age groups affected or the death rate. From the results of the present study where all type 3 pneumococci isolated at the start of the illness were responsible for infections, it would appear unwise to regard such organisms as mere contaminants.

There was in this investigation a slightly increased incidence of types 1 to 8 over the higher types, 57% as compared with 43%. In most similar studies the percentage of type 1 to 8 has been higher. 74% of the pneumococci typed by Humphrey and colleagues (1948) belonged to types 1 to 8. Austrian and Winston (1956) reported that 72% of strains were in this group. In the pneumonias studied by Stuart Harris (1953), the incidence of types 1 to 8 ranged from 61% to 77%.

As with the exception of type 3, the higher types tend to infect younger patients (Heffron, 1939), it would appear that the comparatively low incidence of type 1 to 8 infections is again a reflection of the increased age of the patients in this investigation.

When types 9 and upwards are considered it is seen that 80% of these strains were isolated from patients of 50 years and over, and infection with these types less frequently caused consolidation as compared with infection with the lower type. The illness suffered by the majority of these patients was less severe than that found in a similar age group where the infecting types were of the lower numbers.

In all age groups infection with the lower pneumococcal types was associated with

consolidation. The degree of severity of the illness was associated with the increasing age of the patient.

The predominant infecting types in older patients have been type 3 and the higher types. As these are the types commonly found in the carrier state, it would appear that many of the older patients probably due to a failure of resistance caused by advancing years, have become infected by pneumococci derived from their own respiratory tract, although it is of course possible that these patients were infected from an outside source.

From a consideration of the seasonal incidence of pneumococcal infections, it is seen that there was a predominance of isolations of pneumococci in the late winter and early spring months. It was of interest that with one exception all type 3 pneumococci were isolated in the months November to February. In the study by Straker and colleagues (1939), it was shown that pneumococci were isolated more frequently in the naso pharynx in cold damp weather, and in such conditions there was a tendency for these organisms to spread forwards into the nose and downwards towards the trachea. In the winter months, conditions are then optimum for infection of an endogenous nature, namely an increased

number of pneumococci in the carrier state, and a tendency for the body's resistance to be reduced as the effect of cold and the prevalence of upper respiratory tract infections at this time. If these infections in older people are indeed endogenous, it is apparent that older people who harbour type 3 pneumococci in the naso pharynx run the risk of acquiring a serious illness. In view of the serious nature of pneumococcal infections in older patients, it would appear that consideration of the carrier state is of importance. It would be a marathon task to attempt control of this in the general population. However it has been observed that chronic carriers of pneumococci frequently have pathological conditions of the upper respiratory tract, (Cruickshank, 1933). It would appear that such conditions should not be regarded lightly in older people, as treatment would presumably lessen the possibility of a lower respiratory tract infection.

As the largest number of strains of pneumococci isolated, have been those associated with the carrier state, it is considered that bacteriological examination of the upper respiratory tract in cases of pneumonia could yield interesting and useful information on the possible source of infection.

As the organisms isolated from the blood in all cases of bacteraemia in this investigation was the pneumococcus, it is relevant at this point to discuss this subject.

As other comparable studies have been concerned mainly with pneumococcal infections, the incidence of bacteraemia in the present study has been considered as the percentage of pneumococcal infections.

The percentage of 14.7% found in this series was not an unusual finding. Other comparative reports are 14.8% (Austrian, 1959), 22% (Davis, 1954), 25% (Austrian and Winston 1956), 17% (Ahern and Kirby, 1953), 21% (Gocke and associates, 1949), and 10% (Dowling and Lepper, 1951).

The high incidence of type 3 pneumococci which was observed in general during the investigation was also present in the strains isolated by culture of the blood. Of the ten strains isolated by this means, four were identified as type 3 pneumococci, and 36% of the patients with a pneumonia caused by this type had an associated bacteraemia. Heffron (1939) in a survey of the literature found that type 3 was the least frequent of the first three types to invade the blood. Comparison cannot be made with this survey in view of the small number of

patients concerned in the present series. In more recent studies type 3 has not been found to be the most frequent type isolated from bacteraemic cases. Austrian and Winston (1956) found type 1 a common cause of infection. This type together 2, 5, and 8 was mostly commonly isolated by Thompson and others (1951) and was the commonest type in the study by Dowling and others (1947). In the investigation by Israel and others (1948) there was a variation in the types isolated in the different years of the study. Type 1 was found most frequently in the earlier years but was replaced by type 2 in the later years. Ahern and Kirby (1953) did not isolated any type 3 pneumococci by culture of the blood.

The high incidence of type 3 pneumococcal infections in the present study was associated with the increased age of the patients infected with this type. It was observed that the incidence of bacteraemia rose progressively with age and this association was present with all types isolated. The incidence of bacteraemia in patients of 60 years and over, was five times that of patients under that age.

The mortality rate was high in patients with a pneumococcal invasion of the blood and this was particularly marked when the infecting type was type 3. Three of the four patients with this

form of bacteraemia died. This high mortality associated with type 3 pneumococcal bacteraemia has long been known. In the work of Heffron (1939), it was shown that where the infecting type was type 3 the death rate in bacteraemic patients ranged from 86.1% to 100%.

The serious nature of pneumococcal bacteraemia in pneumonia has been confirmed by other writers. Van Metre (1954) found an increased mortality in such cases particularly where the infecting strains were types 3 and 1. In the study by Dowling and others (1947), five of twelve deaths were in patients with bacteraemia and two of the infecting strains were type 3 pneumococci. Although Thompson and co workers (1951) found an increased mortality associated with bacteraemia, they did not consider type 3 a freqent invader. A high mortality rate associated with culture of pneumococci from the blood was reported by Dowling and Lepper (1951), who found the mortality in bacteraemic patients was 13% as compared with 4% in cases where this complication was not present. There were seven deaths in the study by Anderson and Landsman (1947) and in six of these pneumococci were cultured from the blood. Eight of the twenty two patients who died in the investigation by Anderson and Ferguson (1945) were bacteraemic. Austrian

(1959) and Israel and others (1948) commented on the association of a high mortality and bacteraemia.

It is apparent from the findings in this study, that the presence of bacteraemia, particularly where the infecting type is type 3, in older patients should be regarded as a factor of serious significance.

It is considered that culture of the blood should in included in a study of pneumonia, as it may yield information which could be of assistance to the physician in the early assessment of the case.

It could be of diagnostic value where sputum specimens are not available. This was observed in two cases in the present study where type 14 and type 23 pneumococci were isolated from the blood of two elderly patients who were unable to produce a specimen of sputum.

When infections due to Haemophilus influenzae were considered, it was seen there was a higher proportion of cases regarded as caused by infection with this organism than has been previously reported.

Mulder (1952) stated that he had never isolated a pure culture of unencapsulated Haemophilus influenzae from cases of lobar or

segmental pneumonia. This is in contrast to the present study where in all but one case there was evidence of consolidation. The rarity of pneumonia due to infection with Haemophilus influenzae has been shown by some authors; Schimmer (1959) considered that this type of pneumonia accounted for 0.1% of pneumonias. Of 3,189 cases of lobar pneumonia reviewed by Heffron (1939), 0.2% were considered caused by infection with Haemophilus influenzae. A more recent survey by Crowell and Loube (1954) showed Haemophilus influenzae to be the infecting agent in four out of 3,600 cases of lobar pneumonia. In the study by the Committee of the Antibiotics Clinical Trials of the Medical Research Council (1951), only one of the 267 cases was regarded as due to infection with Haemophilus influenzae. No isolations of Haemophilus influenzae were reported from the investigations of Stuart Harris (1953) Humphrey and colleagues (1948) and Crofton and associates (1951).

These figures are very low when compared with the findings of the present study where Haemophilus influenzae was considered responsible for 9.7% of the infections.

In attempting to find a reason for the high incidence of this type of pneumonia in the present series, it is of importance to consider

possible predisposing factors.

The studies of Mulder and colleagues (1952), Hers and Mulder (1953), and Straub and Mulder (1948), have stressed the importance of epithelial lesions produced by viral infections. These authors considered that necrosis of the epithelium produced by the virus, promoted entry of the haemophilus. In a study of the occurrence of Haemophilus influenzae at post mortem examination, Rosher (1939) considered that invasion of the respiratory by a virus, was conducive to the spread of Haemophilus influenzae from the naso pharynx to the respiratory tract.

In the present study, Para-influenza 1 virus was isolated from one patient. In two other cases it was considered there was serological evidence of infection with Influenza A virus. This diagnosis was based on a rise in virus antibodies.

Haemophilus influenzae is considered an important infecting agent in chronic bronchitis. As six of the fifteen patients in this group were already suffering from the disease, it is probably in these cases that the organism was already present in the bronchial tree and the pneumonia was an exacerbation of a chronic infection.

There is thus a predisposing factor to

infection in only nine of the fifteen cases. This is unusual as infection with Haemophilus influenzae is generally of a secondary nature.

It is of interest to recall the work of Rosher (1939), who showed in post mortem studies of the trachea, that there was an increased incidence of Haemophilus influenzae in the first quarter of the year and considered that cold damp weather favoured the invasion of the lower respiratory tract by Haemophilus influenzae usually harboured in the naso pharynx. In the present study all isolations of pure growths of Haemophilus influenzae were made during the winter months only. It is possible that the hard northern winter may have been a contributing factor in some cases of infection of the respiratory tract with Haemophilus influenzae.

Haemophilus influenzae is a normal inhabitant of the naso pharynx. The carrier rate was studied by Rosher (1939) who found it ranged from 40% to 80% in healthy people. The question therefore arises of whether these organisms were the cause of infection or were commensal in nature.

It was considered they were the cause of infection as the growth obtained on culture was heavy and there was an associated purulence of the sputum.

In 7.0% of cases in this investigation Staphylococcus aureus was considered the infecting agent responsible for the pneumonia. Although this organism was isolated from other patients both in the early and later stages of the illness, it was not considered in some cases to be of clinical significance.

This figure of 7% is comparable with the results of the study by Crofton and others (1951) where staphylococci were isolated from 10 of 110 patients and with one of the investigations of Stuart Harris (1953) in the non influenzal period where a 10% incidence of staphylococcal infections was found. A lower incidence has been recorded in certain other studies. Stuart Harris (1953) in his first investigation in the non influenzal period, found two of 81 cases due to a staphylococcal infection. Seven of the 351 cases of pneumonia studied by Humphrey and associates (1948) were due to staphylococcal infection. An even lower incidence was found in the investigation by the Committee of the Antibiotics Clinical Trials of the Medical Research Council (1951) where, of 267 cases only 2 were caused by infection with the Staphylococcus aureus.

Staphylococcal pneumonia is a frequent and often fatal complication of influenza. (Stuart Harris, 1953; Hers and colleagues, 1958;

Robertson and associates 1958; Walker and others 1958; Maccabe, 1959).

During the present study there was not an outbreak of influenza and this is in accordance with the comparatively low incidence of staphylococcal infections. Virological investigations showed the presence of raised antibody titres to Influenza A in one patient and to Influenza B in another patient. It appeared that chronic bronchitis was more common predisposing agent and was present in six cases infected with this organism. This would indicate the serious danger which infection constitutes to such patients which is emphasised by the presence of two fatal cases in this group.

Scanty and moderate growth of Staphylococcus aureus were obtained from several patients during the later stages of treatment. These were not all considered responsible for clinical infections, which would appear to be unusual as a secondary infection would seen to be a very possible consequence of the presence of these organisms of known virulence.

Phage typing of the staphylococci and nasal swabbing of the patients and staff was not undertaken and it is considered that useful information could be obtained by such a study.

Klebsiella pneumoniae was isolated

infrequently during the investigation, and in only one case was considered to be the cause of pneumonia. Initially this patient suffered from a pneumonia due to a type 3 pneumococcus, and it was considered that infection with Klebsiella pneumoniae took place in hospital. This case demonstrates the advantage of examination of the sputum throughout the pneumonia, as in view of the severity of the nature of infection with Klebsiella pneumoniae it is essential that the correct therapy be given without delay.

Although the incidence of pneumonia due to infection with Klebsiella pneumoniae is low, it is a serious infection and the mortality is high, In the pre antibiotic era, death occurred in the majority of cases. Heffron (1939) reported mortality rates of 63.3 to 90%. The death rate has fallen since the use of antibiotics but still remains at a high level. Jervey and Hamburger (1957) reported a mortality rate of 53%. The patient in the present study recovered.

One strain isolated in this investigation was mouse virulent. Kauffman (1951) maintained that types 1 and 2 which in man produce infections of the respiratory tract can be identified from the higher types by their property of mouse virulence, which is absent in the higher types. However Epstein and Payne (1959) studied

experimental Klebsiella infections in mice and found one type 1 strain which was virulent by the intraperitoneal route. However the value of mouse inoculation cannot be disregarded on such scanty evidence.

Strains of Klebsiella pneumoniae were isolated from some cases in the present series in the later stages of the illness, but were not considered to be of clinical significance. Most probably these strains which were penicillin resistant were the result of colonisation of the respiratory tract after treatment with penicillin. Weiss and colleagues (1956) found most strains isolated from patients following penicillin treatment were of the higher types.

In view of the severity of the infection and the mortality assocated with it, it is essential in infections caused by Klebsiella pneumoniae, that the organism be recognised and the appropriate therapy instituted without delay. Jervey and Hamburger (1957) and Limson and others (1956) stressed the importance of a Gram film in the identification of the organism.

Such an examination applies to all types of pneumonia, and this examination is a useful aid to the early identification of the nature of the bacterial infection of the sputum.

As no pathogenic bacteria were isolated from just over a third of the cases in this investigation, it would seen advisable to study these in some detail in order to find possible reasons for this apparent failure to establish a bacterial aetiology in so many cases of pneumonia.

A finding of this nature is not new. Stuart Harris (1959) commented on the appearance of this group of bacteriologically undiagnosed pneumonias in the more recent studies of pneumonia.

In the study by Crofton and colleagues (1951) 47% of the cases were not diagnosed bacteriologically. Grist and others (1952) commented on the high proportion of cases 27%, from which no pathogenic organisms were isolated. 15% of the cases studied by Humphrey and co workers (1948) and 20% of the cases in the investigation by the Committee of the Antibiotics Clinical Trials of the Medical Research Council (1951) were not diagnosed bacteriologically.

Previous antibiotic treatment may mask the infecting bacteria and this particularly applies to the pneumococcus which is sensitive to many antibiotics. Crofton and colleagues (1951) considered that several cases of pneumococcal pneumonia were undiagnosed for this reason. In the present study this was a possible explanation

of failure to isolate pathogenic bacteria in just under half the cases.

A profuse growth of Monilia was isolated from three cases and a profuse growth of Bact coli from one case in the untreated group. This finding raises the question of treatment which had not been reported. Although Monilia may be found in the sputum of healty people, it was considered that the amount of growth in these cases was in excess of normal. It was not considered that the Bact. coli isolated were the primary cause of the pneumonia in view of the patient's response to penicillin.

It was observed in the investigation that culture of the sputum was a superior method of isolation of pathogenic bacteria than culture of laryngeal swabs. From four patients, only laryngeal swabs were received, and it is considered in these cases the possiblity of isolation of pathogenic bacteria was diminished for this reason.

In addition, in these cases where only laryngeal swabs were examined, mouse inoculation was not performed, and this was found to be an effective method of isolation of pneumococci in the present study. Fourteen strains of pneumococci were isolated by this method only. Humphrey and others (1948) considered the isolation rate of

pneumococci could be increased by 35% by the use of this technique.

The type of sputum specimen was of importance. When the specimens were received, the type was recorded and on analysis of the results, it was observed that in two of the cases where no bacterial pathogen was isolated, the specimen was salivary in nature and unlikely to have yielded information on the inflammatory process present in the lower respiratory tract. The examination of a true specimen of sputum cannot be overemphasised. Humphrey and colleagues (1948) stressed the importance of the type of specimen. The suitability of the specimens in their investigation was assessed by the medical staff or sister before dispatch to the laboratory.

The organisms which were isolated most frequently in this group were Neisseria catarrhalis and Strept. viridans. There was a predominance of older people in this group and the possible pathogenicity of these organisms to such patients should be considered. Several patients were very elderly and it may be that the impairment of the body's defences allowed these organisms to gain access to the lower respiratory tract with a resulting inflammatory reaction. Scadding (1952) has described a type of pneumonia

which he has named 'aspiration' pneumonia. The nature of the aspirate is the endogenous secretion of the respiratory tract which is not expelled as a result of failure of the defence mechanism of the bronchi. This failure which permits stagnation of the secretion, may occur in old age where weakening of the respiratory muscles may lead to difficulty in the expectoration of the secretions of the bronchial tract. In such cases pathogenic bacteria are not cultured from the sputum but the bacterial flora is made up of organisms usually considered of a non pathogenic nature, for example N. catarrhalis, and Strept. viridans.

The possibility of infection with organisms normally non pathogenic was discussed by Crofton and others (1951). Previous damage to the bronchial epithelium could facilitate infection and it is considered that this may have occurred in two cases in the present study where large numbers of Strept. viridans and N. catarrhalis were associated with purulence of the sputum. In both cases there was serological evidence of previous viral infection and no pathogenic bacteria were isolated. Hers and Mulder (1953) considered that necrosis of the epithelium by the influenza virus promoted entry of pathogenic bacteria, and it is possible that such a lesion

has aided infection in these cases. This was most suggestive where N. catarrhalis was isolated, as the intracellular distribution of the organisms indicated an acute inflammatory process.

From this study it would appear that the usual practice of ignoring 'non pathogenic' organisms should be revised, particularly where debilitated patients are concerned. It is considered that the age and general condition of the patient should be taken into account when the significance of organisms present in the sputum is assessed.

It can also be seen from this study that in a bacteriological investigation of pneumonia, an associated virological study is necessary. Apart from the primary nature of such infections, the possibility, as has already been discussed, of viral lesions predisposing to infection with organisms usually regarded of low pathogenicity, should be considered.

To summarise, it appeared that many of the cases in this group could be accounted for. Some were possibly missed bacterial pneumonias, either as a result of previous antibiotic treatment which had masked the infecting organism, or a result of an incomplete bacteriological examination. In some cases the probable infecting agent was a

virus. In a few cases it was possible the infecting organism was one normally of low pathogenicity which had gained access to the lower respiratory tract as a result of a failure of the host's defences. It was considered that possible predisposing factors were old age and viral infections.

Replacement of sensitive flora by fungi and Gram negative bacilli may occur during or after antibiotic treatment. In the present study this took place in 63 cases (43%). With the exception of one strain of Pseudomonas pyocyanea these organisms were of a harmless nature and their presence did not give rise for alarm. The strain of Ps. pyocyanea was isolated from tracheostomy specimens in the terminal stages of a pneumococcal pneumonia. In such cases there is difficulty in assessing the pathogenic nature of such an organism isolated in the later stages of a fatal illness. Although it may be assumed to be the infecting agent, its continued presence in large numbers may be the consequence of continued therapy which has removed all but the most resistant organisms.

It is frequently difficult to determine the significance of Gram-negative bacilli isolated during the treatment of respiratory infections. It is considered that examination of a Gram film

can be of assistance, as by this means the degree of inflammatory reaction can be estimated. In a minority of cases there is marked purulence associated with the presence of Gram-negative bacilli only, in large numbers. It is considered that these cases are of more possible significance than those where the pus cells are fewer and the bacterial flora is mixed. In the case in the present study where Ps. pyocyanea was isolated terminally, examination of the Gram film showed large numbers of pus cells and Gram-negative bacilli.

There are several reports of pulmonary infections by Gram-negative bacilli subsequent to antibiotic treatment. Weinstein (1955) reported cases of this type of pneumonic infection in patients with respiratory poliomyelitis who had received prophylactic antibiotic treatment. In a study by Barach and colleagues (1952), on the effect of antibiotic treatment on chronic respiratory infections, several severe superinfections with Proteus and Pseudomonas aeruginosa were encountered and these organisms were regarded as the infecting agent in some fatal cases. McCurdy and Neter (1952) and Haffner and associates (1950) studied this problem in young children. In these investigations it was observed that after antibiotic therapy the normal

flora of the upper respiratory tract was frequently replaced by Gram-negative flora.

Antibiotic sensitivity

An important aspect of this investigation was the study of the antibiotic sensitivity of the pathogenic organisms isolated.

By a study of results obtained by the plate dilution method it was hoped to learn at least two things. One was the exact degree of sensitivity of the organism to the drug, and if it was isolated at later examinations, any change in the sensitivity As this was an *in vitro* sensitivity, the results would require to be correlated with the clinical response to the drug. The control organism was a standard sensitive *Staphylococcus aureus* and thus when an organism was regarded as resistant, it was so when compared with the sensitivity of the standard organism.

The most interesting finding which emerged from this investigation was the appearance of certain strains of pneumococci which showed a decreased sensitivity to penicillin.

Pneumococci have always been regarded as extremely susceptible to the action of penicillin. Austrian (1957) considered it was not necessary to test the sensitivity of pneumococci isolated from cases of pneumonia.

The sensitivity pattern which emerged in the present investigation was therefore surprising and gave rise to concern. No previous studies

had reported loss of sensitivity to penicillin by pneumococci. Hoffman and Volini (1947) considered penicillin resistant pneumococci a possible reason for failure of response to oral penicillin in the treatment of pneumonia, but in these cases the organisms were not isolated and sensitivity tests were not performed.

The in vitro sensitivity of pneumococci to penicillin was studied by Jackson and colleagues (1950). In this study the highest concentration of penicillin which would inhibit growth of the pneumococcus was 0.01 units per ml. A similar investigation was undertaken by Jones and Finland (1957). The compared their results with the earlier workers and found no significant change had taken place in the sensitivity of the pneumococcus to penicillin.

At the start of the investigation, pneumococci were identified by their morphology, colonial appearance, solubility in sodium desoxycholate, optochin sensitivity and inulin fermentation. Certain strains of pneumococci which were typical colonially and morphologically were found to lack the properties of bile solubility, optochin sensitivity, and inulin fermentation. Although this was found in a few penicillin sensitive strains, it was a constant feature in those strains which were resistant to penicillin.

Because of these findings it was decided to

include mouse inoculation in the range of tests as it was considered the property of mouse virulence was an indication of the significance of the organism. As it was also considered that determination of the type of pneumococcus was of great value, this investigation was added to the range of tests.

In the present study, the identification, and the assessment of the significance of penicillin resistant bacteria which had the colonial and morphological characteristics of pneumococci but lacked the biochemical reactions of these organisms, presented difficulties. If the possession of the properties of bile solubility, optochin sensitivity, and inulin fermentation is the criterion necessary for the identification of pneumococci, then the organisms in the present study cannot be regarded as pneumococci. On the other hand as they were capsulated, and when facilities for mouse inoculation became available, were shown in many cases to be mouse virulent, there seemed to be a strong indication that they were pneumococci which had been altered by the action of penicillin. Useful information was gained from the study of <u>in vitro</u> resistance in pneumococci. Here it was shown that as pneumococci grew in increasing concentrations of penicillin, the properties of

bile solubility, optochin sensitivity and inulin fermentation were lost and by this means it was possible to produce capsulated pneumococci which were bile insoluble, optochin resistant and failed to ferment inulin. It was found that pneumococci which became resistant *in vitro*, retained the capsule until growing in concentrations of 0.8 units to 1.0 units per ml., while the majority of strains lost the biochemical reactions during growth in lower concentrations of penicillin. In some strains with *in vivo* resistance, the capsule was retained when the inhibiting concentration of penicillin was greater than 1 unit per ml. It is not however intended to make a direct comparison between the two forms of resistance as the nature and circumstances of each are widely different, but it is considered there is a similarity between the findings which may explain in part the unusual finding of capsulated pneumococci with a reduced sensitivity to penicillin, and without the accepted biochemical reactions of the organism. It is not considered that a comparison of the property of mouse virulence in strains with *in vivo* and *in vitro* resistance can be made, as the rapid loss of this property in strains with *in vitro* resistance was regarded as possibly due to the frequent subcultivation which was required in this experiment.

Alteration in character of an organism after growth in a medium containing penicillin is not a new observation. In 1940 Gardner described the production of a flocculent growth of Clostridium welchii and pleomorphism in strains of Streptococcus pyogenes when these organisms were grown in the presence of penicillin. It has been shown that strains of Staphylococcus aureus when grown in increasing concentrations lose their morphological appearance and become Gram-negative and bacillary in form (Klimek and colleagues, 1948; Bellamy and associates 1948). Bellamy and associates (1948) demonstrated that the organisms had feebler fermentative powers than the original strain, failed to grow in 6.5 NaCl, and did not reduce nitrates. Staphylococcus aureus with a moderate degree of induced in vitro resistance to penicillin regained sensitivity when grown in a penicillin free medium. (Todd and colleagues, 1945a; Klimek and associates, 1948). In the present study it was found that the level of penicillin resistance induced in pneumococci remained constant after numerous transfers in a penicillin free medium. This is in accordance with other workers in this field (Todd and colleagues, 1945b; McKee and Houck, 1943).

Pneumococci which were resistant to

penicillin were in two groups. In the first group were organisms which possessed primary resistance and were isolated at the start of the illness from patients who had not received treatment with penicillin. These organisms retained the typical morphological and colonial appearance of the pneumococcus.

In the second group were organisms which had acquired resistance to penicillin and were isolated in most cases from patients who had received treatment with penicillin and from whom penicillin sensitive pneumococci were isolated at the start of the pneumonia. One case was placed in this group as there was a history of a recent pneumococcal infection which had been treated with penicillin. There were two varieties of organisms which showed acquired resistance to penicillin. The first were morphologically and colonially typical pneumococci. The second type were acapsulated Gram positive diplococci smaller morphologically and colonially than typical pneumococci and were bile insoluble, optochin resistant, and usually fermented inulin. It was possible by subcutaneous inoculation of a mouse to reverse a few of these strains to type specific pneumococci with an increased resistance to penicillin.

Where the resistance to penicillin was

primary, the inhibitory concentration of penicillin ranged from 0.5 to 4 units per ml.

From the severe nature of the infection suffered by one patient in this group, it was apparent that penicillin resistant pneumococci may possess a marked degree of virulence. This patient had a fulminating pneumonia with bacteraemia and died 12 hours after admission to hospital. The infecting pneumococcal type in both sputum and blood was type 3. It cannot be assumed that penicillin resistance was the factor responsible for virulence, but it is of interest that of all the type 3 infections, this case ran the most rapidly fatal course.

It was observed that type 3 pneumococci were inhibited by a higher concentration of penicillin than the other two types identified.

Unfortunately the one other pneumococcus which was inhibited by a high concentration of penicillin was not typed. As there was delayed radiological clearing in this case, knowledge of the type would have been of interest, in view of the similar findings with type 3 pneumococcal infections.

With such a small number of cases it is not possible to make more than tentative suggestions. However it would seen that type 3 infections did adversely affect the progress of

the illness, and were associated with high inhibitory concentrations of penicillin.

In the group where acquired resistance appeared during treatment with penicillin, and the pneumococci retained their typical colonial and morphological appearance, one case was of particular interest. There was a previous history of a pneumococcal pneumonia some weeks before and the current illness was complicated by the presence of a sarcoma and chronic bronchitis. Although there was no doubt that the presence of the sarcoma was an important contributing factor in the fatal outcome of the illness, the pneumococcus isolated was responsible for an acute inflammatory process.

The question arises if the pneumonia was a fresh infection from an outside source or a reinfection with the same organism which was responsible for the first illness. As neither strain was typed it is not possible to determine which form of infection occurred. However in view of the history, it would seem pertinant to consider the possibility of the patient being a convalescent carrier. The presence of this carrier state was recognised in the pre antibiotic era. Cruickshank (1933) isolated type 1 and 2 pneumococci from patients twenty six months after recovery from lobar pneumonia due to infection

with these types. It would seem that a similar study in the present day could yield interesting information concerning the presence of carriers, and the sensitivity to penicillin of pneumococci isolated from patients who had received treatment with this drug. In the remaining patients in this group, it appeared that the appearance of penicillin resistance in the course of treatment, with the exception of some cases where radiological clearing was delayed, did not have an adverse effect on the progress of the illness. As only one pneumococcus in this series was typed, it was not possible to say if the strains isolated at later examinations were the same as those isolated at primary examination. The one type identified was a type 3 pneumococcus.

Jones and Finland (1957) attempted to discover if there was any relation between the degree of sensitivity to penicillin and the infecting type of pneumococcus, but were unable to find a connection.

In the present study, although the numbers were small, it was seen that of the pneumococcal types isolated, type 3 pneumococci were inhibited by the highest concentration of penicillin.

The other form of acquired resistance was observed in Gram positive diplococci which were resistant to penicillin.

These organisms were isolated during the later stages of pneumococcal pneumonias where the patients had been treated with penicillin. It was considered that they might be related to the pneumococcus for two reasons. One was their appearance in the sputum in the later stages of the illness. The other was the resemblance betweenthem and the organisms produced by the action *in vitro* of penicillin on pneumococci. Both were penicillin resistant rather small acapsulated Gram-positive diplococci growing in diminutive colonies, insoluble in sodium desoxycholate, insensitive to optochin, frequently failing to ferment inulin and avirulent to mice.

It was therefore decided to explore the possibility of transforming into typical pneumococci thos Gram-positive penicillin resistant diplococci which were isolated from some patients with pneumococcal pneumonia, in the later stages of their illness.

Previous passage experiments using the intraperitoneal route had been unsuccessful in restoring virulence to avirulent pneumococci. However Griffith (1928) using subcutaneous inoculation in the reversing rough strains of pneumococci into smooth capsulated strains.

It was therefore decided to apply this method since the object in view was similar to

Griffith.

As the production of a smooth capsulated pneumococcus was achieved in some experiments, the assumption was that penicillin *in vivo* produced a rough form of pneumococcus.

It has long been redognised that pneumococci undergoing subcultivation may become altered. Loss of the capsule is associated with the appearance of the rough form of colony. Work on the transformation of the pneumococcus has shown there are variations in this type of colony from very rough described by Ephrussi Taylor (1951) to intermediate from between smooth and rough described by McLeod and Krauss (1947,1950). Austrian (1953) using a selective cultural technique, produced a filamentous capsulated form of pneumococcus. These are however *in vitro* changes and there few reports of changes in pneumococcal morphology occurring *in vivo*.

In 1910 Rosenow described a study of seven cultures isolated from endocarditis. He considered they were modified pneumococci which fermented inulin but grew atypically on culture. Masters and colleagues (1958) described optochin sensitive organisms which grew in small colonies and were considered to be 'rough' pneumococci.

Two factors were features of the successful

experiments. One was the need to employ as the transforming strain the same type of pneumococcus as had been isolated at the start of the pneumonia. When such a strain was not used, although Gram-positive diplococci were isolated from the subcutaneous lesion in some experiments, it was not possible to type them and they remained avirulent to mice even after passage. This varied from the results of Griffith (1928) who was able to cause pneumococci to assume the capsule of a different type.

The other feature was the success was more frequent when the strain of pneumococcus responsible for the pneumonia, was of a virulent nature. The types in these cases were types 3 and 23. This latter type although not one of generally recognised virulence, had in this case caused a bacteraemic condition which could be regarded as an indication of its virulence on this occasion.

There can be no doubt that these atypical resistant strains isolated from patients during the later stages of pneumonia would not have been recognised if the sputum had not been studied throughout the illness. Their appearance was not typical of a pneumococcus and they would have been possibly been labelled as a Strept. viridans without a previous knowledge of the organisms isolated at the start of the pneumonia.

In this trial, examination of only a maximum of four specimens of sputum was done. It would be of interest if daily examination of the sputum could be undertaken. By this the morphological and biochemical changes occurring in the pneumococcus could be studied in detail and correlated more closely with the changes found in vitro.

Of the experiments performed, not all were successful in producing transference of the capsular type. The results of Griffith (1928) showed that not all transformation experiments were successful. In the present study the cultural techniques may have been at fault. The cultures were cultured overnight but perhaps a shorter incubation time would have produced a more effective result.

Success might have been more frequent if the original infecting strain of pneumococcus from the patient concerned had been used as the transforming strain. The virulence of this strain could be retained by passage through successive mice.

General observations on penicillin resistance in pneumococci

Extreme sensitivity to penicillin has so long been regarded as a property of the pneumococcus, that a statement concerning

possible resistance to the drug must be met with scepticism. Indeed the initial sensitivity tests were repeated without delay such was the degree of unbelief with which they were regarded.

There is a likeness between these findings and similar results obtained with the gonococcus. Pneumococci and gonococci were regarded as organisms which were highly susceptible to penicillin and which showed no signs of becoming resistant to its action. This picture was changed by reports of gonococci which did not respond to treatment with penicillin. Curtis and Wilkinson (1958) measured the sensitivity of 302 strains of gonococci. 19.5% of these were inhibited by 0.125 to 0.5 units of penicillin per ml., and failures of treatment occurred in this group. No failure was found when the inhibiting concentration was below 0.03 units per ml. A similar study was done by Cradock Watson and colleagues (1958) who found resistance to treatment greater in strains where the inhibitory concentration of penicillin was 0.128 units per ml.

From a consideration of the few cases where the pneumococcus was typed, it seemed that virulent strains of pneumococci tended to become resistant to penicillin. This was observed where the resistance was primary where of four

strains identified two were type 3 pneumococci and one was a type 8 pneumococcus. It was not possible to identify most of the pneumococci which acquired resistance but retained their typical appearance, but it was of note that the one strain which was typed was a type 3 pneumococcus. Association with virulence was seen, where acquired resistance occurred in pneumococci which lost their typical appearance, by the reversal of two avirulent organisms to capsulated pneumococci of the virulent types responsible for the original infections. It is realised that the numbers in this study are too small to be of statistical significance but it was considered the findings were of interest in view of other statements on penicillin resistance in type 3 pneumococci.

In the studied by McKee and Houck (1943), Rake and colleagues (1944), and Schmidt and Sesler (1943) of induced resistance to penicillin, it was shown that type 3 pneumococci acquired resistance to penicillin more rapidly than other types studied. A similar observation was not made in the present study of <u>in vitro</u> production of penicillin resistance.

Hotchkiss (1951) stated that type 3 smooth strains of pneumococci can give rise spontaneously to a very small number of smooth penicillin resistant strains.

The spontaneous mutation of penicillin resistant variants could be a possible explanation of the development of penicillin resistance in a type 3 pneumococcus treated with penicillin. This could apply to the one case of acquired resistance where the infecting type was a type 3 pneumococcus. Where the resistance was primary and no penicillin had been received, it would appear possible that the infection was an exogenous one. Type 3 pneumococci are found in 10.5% of healthy carriers (Heffron 1939). It may be as a result of the widespread use of penicillin that certain of these strains have acquired resistance to penicillin.

The possibility of penicillin resistant pneumococci in the carrier state arose in one case of acquired resistance where there was a relapse of pneumococcal pneumonia.

It is apparent from this study that for an understanding of penicillin resistance in pneumococci, typing of the organism is necessary. Unless this is done the significance of resistance developing <u>in vivo</u> cannot be estimated.

Finally this investigation demonstrates that it is essential for the sensitivity of the pneumococcus to penicillin to be estimated. It

can no longer be assumed that this organism is sensitive to penicillin.

Resistance to streptomycin was observed in the majority of strains of pneumococci isolated. In addition to the strains of pneumococci which were resistant at the start of the pneumonia, it was apparent from examination of a typable strain which was present on more than one occasion, that resistance to streptomycin was quickly acquired. When the pneumococcal types which showed resistance were considered, it was seen that there was a fairly wide range of types affected. The high incidence of streptomycin resistant strains would indicate that this antibiotic is not the drug of choice in pneumococcal infections. By some physicians the administration of streptomycin in any type of acute respiratory infection is considered inadvisable, until the possibiiity of infection with the tubercle bacillus has been eliminated, in view of the rapidity with which this organism acquires resistance to streptomycin.

The presence of streptomycin resistance in pneumococci was taken advantage of by Masters and colleagues (1958) who by incorporating 2 mcg. per ml in the culture medium obtained a good selective medium for the isolation of pneumococci.

Although most of the strains were sensitive to tetracycline, there were some where

the sensitivity was reduced. It may be considered that this degree of resistance is of doubtful significance but it is of interest to find this range of sensitivity in an organism which is considered sensitive to tetracycline.

Apart from its importance in acute respiratory infections, the pneumococcus is of significance as an infecting agent in chronic bronchitis. Long term therapy with this drug is frequently administered in an attempt to prevent damage to the lung tissue by infection. The appearance of resistance to tetracycline by pneumococci is therefore of great importance. It might be considered that the appearance of a degree of resistance is an indication of the extent of this form of treatment in recent years.

The pneumococcal types which showed reduced sensitivity to tetracycline were varied, and it was of interest that they were types associated with the carrier state and infection in chronic bronchitis.

In the present study there was evidence of acquired resistance in typable strains isolated at later examinations. Jones and Finland (1957) described a case of pneumococcal pneumonia in which the organism showed an <u>in vitro</u> increase in resistance to tetracycline. The inhibitory concentration rose from 0.2 mcg. to 6.3 mcg per

ml., from the first to the fifth week. These authors suggested that pneumococci sequestered within large areas of necrosis and suppuration may acquire resistance during the course of treatment.

Recently Parker and colleagues (1962) commented on the appearance of tetracycline resistant strains of Strept. pyogenes in various parts of Great Britain. 12% of 921 strains tested by the authors were resistant, some of these being inhibited by concentrations of between 50 and 100 mcg. per ml. Like the results in the present study these findings were unexpected, and would seem to indicate that changes may occur in the sensitivity pattern where organisms are exposed to antibiotics over a prolonged period. The susceptibility of an organism to an antibiotic can no longer be assumed but must be confirmed in the laboratory.

Approximately 75% of the strains of Staphylococcus aureus isolated were resistant to penicillin. From a review of the literature from many countries including the United States and England, Finland (1955) concluded that almost three fourths of all strains of coagulase positive staphylococci in hospital patients are penicillin resistant. A high proportion of penicillin resistant strains isolated in the later stages

of the pneumonia appeared to be associated with hospital infection rather than acquired resistance in a previously sensitive strain. Barber (1953) showed that the epidemic hospital strains were able to displace the strains which the patient carried in the nose. In this study she demonstrated that 10% of patients on admission carried penicillin resistant strains whereas 58% did so on discharge from hospital.

There was evidence that resistance might have been acquired by some strains in the course of treatment. As phage typing was not done it is not possible to say if these strains persisted throughout. In a study by Goslings and colleagues (1959), there appeared to be an increased resistance to antibiotics in strains isolated after admission to hospital but phage typing of these strains showed that the type was different to that isolated at primary examination.

Resistance to tetracycline was observed in half the strains of Staphylococcus aureus isolated in this study. The review by Finland (1955) indicated that the incidence of tetracycline resistant strains was lower than this figure. The higher incidence now found may be a measure of the increasing use of tetracycline as a therapeutic agent in the intervening years. From the appearance of tetracycline resistant strains

in patients during their stay in hospital, it could be assumed that the hospital strains were resistant to the drug. There was little evidence of acquired resistance occurring during treatment but without identification of the phage type it cannot be confirmed if this did take place.

The sensitivity pattern of streptomycin was very similar to that of tetracycline. Approximately half the strains were resistant to the drug and all strains isolated from the first time during the later stages of the illness were resistant. There was little evidence of resistance appearing during treatment.

From the presence of resistance to penicillin, streptomycin, and tetracycline in all strains of Staphylococcus aureus isolated for the first time at later examinations, it might be concluded that this was the pattern of resistance of the organisms prevalent in the hospital environment. It is therefore surprising that 32% of these strains were not responsible for a secondary infection, as in the opinion of Barber (1962) such strains are of high virulence having survived attack by both body defences and antibiotics.

The three antibiotics discussed, penicillin, tetracycline, and streptomycin have been commonly used in the treatment of staphylococcal

infections. In view of the degree of resistance found in many of the strains it would appear desirable to determine the sensitivity of the organism before commencement of treatment. For a full understanding of the apparent development of resistance to these drugs, it is essential that phage typing be undertaken.

In contrast to the resistance observed with penicillin, tetracycline, and streptomycin, all strains of Staphylococcus aureus isolated during the investigation were sensitive to chloramphenicol and novobiocin.

Initially all strains were sensitive to the action of erythromycin. However resistance appeared to develop in one case. The level of the inhibitory concentration of erythromycin rose rapidly in the course of two days treatment. Finland (1955) remarked on the steady increase in resistance to erythromycin, after exposure to the drug. In view of these findings, it would be advisable where the patient is receiving erythromycin, that the sensitivity of every strain of Staphylococcus aureus isolated be estimated in order that resistance is detected without delay.

When the sensitivity of Haemophilus influenzae to penicillin was considered it was seen that the strains isolated were divided into two main groups, a smaller one where the organisms

were highly sensitive to penicillin and a larger one where they were highly resistant to the action of the drug. With the exception of one strain isolated for the first time on the tenth day, all the sensitive organisms were isolated at the start of the pneumonia. As all strains of Haemophilus influenzae isolated in the investigation were unencapsulated, the isolation of these sensitive strains is an unusual finding.

Fleming (1929) considered Haemophilus influenzae insensitive to penicillin and advised incorporation of penicillin in culture medium in order to facilitate isolation of Haemophilus influenzae from mixed cultures.

A study of in vitro sensitivity of Haemophilus influenzae was made by Finland and Wilcox (1950). Of 30 strains examined 7 were inhibited by concentrations of penicillin of up to 0.5 units per ml. Three of these were inhibited by concentrations of 0.1 to 0.2 units per ml. which is higher than the levels of 0.03 and 0.06 units per ml. found in the present investigation.

In the investigation by Gordon and Zinneman (1945), the lowest concentration of penicillin which inhibited growth was 1 unit per ml. Stuart Harris (1953) considered that Haemophilus

influenzae was inhibited by concentrations of penicillin ranging from 0.1 to 10 units per ml.

From the results of the present study it would appear that there are rough strains of Haemophilus influenzae which are highly sensitive to the action of penicillin. The incorporation of penicillin in culture medium would result in failure to isolate such strains and the widespread use of such media may be the reason why no previous reports of strains of Haemophilus influenzae highly sensitive to penicillin have been recorded.

In two cases where penicillin sensitive strains were isolated on the first day, penicillin resistant strains were isolated on the second day. In a third case second day examination was negative but penicillin resistant strains were isolated on the fifth day. There are two possible explanations. There may have been a rapid acquisition of resistance, a finding which has not been reported previously. As it is not possible to identify rough strains of Haemophilus influenzae, this theory must remain unconfirmed. Another explanation is that the organisms isolated at later examinations were derived from the patient's naso pharynx or from the environment.

Although most strains of Haemophilus

influenzae were sensitive to tetracycline, two strains showed in vitro resistance to this antibiotic. This finding would seen to be of great importance in view of the frequency with which tetracycline is used as maintenance therapy in chronic bronchitis. Although Haemophilus influenzae is an infrequent cause of pneumonia, it is a frequent infecting agent in chronic bronchitis, and is responsible for acute infective episodes which destroy lung tissue and impair pulmonary function. Using the disk diffusion method for determination of the antibiotic sensitivity, Murdoch and colleagues (1959) considered that during treatment with oxytetracycline, four out of thirty five patients produced drug resistant strains of Haemophilus influenzae in the course of treatment. It would seem advisable therefore before the start of treatment and during the period of preventive therapy, to test the sensitivity of the organism as it would appear that certain strains are not completely sensitive to the action of this antibiotic.

The majority of strains of Haemophilus influenzae isolated were sensitive to streptomycin, but there were a few strains which were inhibited by a concentration which was higher than previous writers have reported. The range of streptomycin

causing inhibition of growth in the study by Finland and Wilcox (1950) was from 1.6 to 3.1 mcg. per ml. Stuart Harris (1953) considered the inhibitory concentration was from 1.0 to 10 mcg per ml. An inhibitory concentration of 32 mcg. per ml was found in two strains isolated in the present study. Streptomycin was considered by Kirby (1955), and Crowell and Loube (1954), a possible drug in the treatment of pneumonia caused by Haemophilus influenzae. It would appear from the present investigation that although in most cases this is so, there are resistant strains, and preliminary sensitivity testing is advisable.

All strains of Haemophilus influenzae were sensitive to chloramphenicol. It would however be inadvisable to use this drug in the treatment of respiratory infections in view of the possible occurrence of a blood dyscasia which is a side effect of this antibiotic.

The high resistance to penicillin present in all strains of Klebsiella pneumoniae isolated during this investigation is in agreement with previous studies. Epstein (1959) and Jervey and Hamburger (1957) found all strains resistant to penicillin.

The sensitivity to tetracycline indicated a higher degree of resistance than had been

reported previously. Epstein (1959) found all strains sensitive and Limson and colleagues (1956) considered one of twelve strains examined resistant.

One strain was resistant to streptomycin. In the studies by Jervey and Hamburger (1957) all strains examined were sensitive. Limson and others (1956) considered one strain resistant to streptomycin.

There were three strains which showed resistance to chloramphenicol which compares with the findings of Jervey and Hamburger (1957). Epstein (1959) considered all strains were sensitive to Chloramphenicol.

It would appear from the present study that the majority of strains of Klebsiella pneumoniae were sensitive to streptomycin, chloramphenicol, and tetracycline. There were exceptions which would indicate the advisability of determining the sensitivity of the organisms before treatment is commenced. Although the sensitivity of the organisms to streptomycin would appear to be a factor influencing the choice of this drug, rapid resistance to streptomycin is readily acquired. This was observed by Finland and colleagues in 1946.

Factors influencing the isolation rate of pathogenic organisms

It was apparent from the findings of this

study, that for full investigation of the bacterial flora of the sputum, it was necessary to employ certain selective techniques.

From a comparative study of untreated and homogenised sputum, it was evident that culture of the latter type of specimen yielded the greater number of pathogenic organisms and if this procedure had not been undertaken, many of the cases would not have been diagnosed bacteriologically. This finding is in agreement with May (1952, 1953a) who demonstrated that pathogenic bacteria were irregularly distributed in the sputum. To overcome this he advocated homogenisation of the sputum and a technique to produce this effect was devised by Rawlins (1953), who used pancreatin as a homogenising agent. A later study by May (1954) showed the effectiveness of this method in producing a regular distribution of pathogenic organisms in the sputum.

In the present study, the sputum was treated by shaking with water and beads, a method which was considered equally as effective as homogenisation with pancreatin. This has been demonstrated in the earlier part of this thesis. The production by homogenisation with water, of a specimen suitable for intra peritoneal inoculation of mice, was considered to be an

additional advantage of this method.

The only selective medium employed in this investigation was heated blood agar. This was used for the isolation of Haemophilus influenzae and omission of this medium would have resulted in a failure to identify some strains of this organism.

Previous investigators have commented on the value of a selective medium for the isolation of Haemophilus influenzae (Humphrey and colleagues 1948; Stuart Harris and associates, 1953).

There was no doubt that culture of pneumococci in an atmosphere of 10% carbon dioxide enhanced the growth and facilitated recognition of the organism. Humphrey and colleagues (1948) considered that culture under these conditions improved the isolation rate of pneumococci. In the present investigation there were two strains of pneumococci which grew only in the presence of carbon dioxide. It is of interest that these patients were classified as severe chronic bronchitics. It is possible there is a relationship between this finding and the poor pulmonary ventilation present in many chronic bronchitic patients. Fleming in 1941 reported a strain of pneumococcus which grew only under the influence of carbon dioxide and advised the use of such cultural conditions for the

isolation of pneumococci.

A moist atmosphere was found to improve the growth of pneumococci. In this investigation the method employed to produce carbon dioxide was the action of hydrochloric acid on the chalk. A certain amount of moisture appeared in the container and it was observed that the amount of growth was in proportion to the degree of condensation. To further this a jar of water was placed in the container. The enhancing effect of a moist atmosphere on the growth of pneumococci was observed by Masters and colleagues (1958).

It was obserbed that mouse inoculation was a highly efficient method of isolation of pneumococci, and by the employment of this technique the isolation rate of this organism could be considerably increased. Fourteen strains were isolated by this method alone and it is considered probable that certain cases in the early part of the trial, were not diagnosed bacteriologically as facilities for mouse inoculation were not then available. Omission of this technique in the largest part of the study by Crofton and colleagues (1951), was regarded by these authors as a likely cause of failure to isolate pneumococci from probable cases of pneumococcal pneumonia . Humphrey and others

(1948) considered that the isolation rate of pneumococci could be increased by 35% by the addition of mouse inoculation to the range of tests.

Of the other pathogenic bacteria isolated by mouse inoculation, all were found on direct culture of the sputum and the property of mouse virulence did not appear to be significant. The pneumonias caused by a few strains of mouse virulent Haemophilus influenzae and Staphylococcus aureus were not of marked severity and the patients made uneventful recoveries.

By the criterion of Kauffmann (1951) who considered that mouse virulence was a property of strains of klebsiella which are the cause of respiratory infections, the one isolation of Klebsiella pneumonia in this series could be regarded as of significance. It proved to be of clinical significance in this case as it revealed a new infection probably originating from the hospital environment in a patient who was recovering from a pneumococcal pneumonia.

When the results of culture of sputum and laryngeal swabs were considered it was apparent that culture of the sputum was the more effective method of isolation of pathogenic organisms. If it can be assumed that the technique of swabbing the larynx was beyond reproach, it

would seem advisable to study further the bacteriological methods employed in examination of the swabs. The swabs were plated on blood agar, and heated blood agar; this was the only examination undertaken, it would seem advisable to make a fuller bacteriological study. In the study by Austrian (1959), the swabs were incubated in peptone broth and the culture inoculated on blood agar and into a mouse. An improved isolation rate of pneumococci and Haemophilus influenzae from the nose and throat swabs was obtained by Masters and colleagues (1958) by the use of Pike's medium which contains crystal violet and sodium azide in blood agar. The culture of laryngeal swabs is therefore a subject which should be studied further as this specimen may be the only one available from a case of pneumonia. Difficulty in coughing is frequently encountered in pneumonia as a result of pain, and in old age where debility may impair the expectoration of the bronchial secretions.

It was observed in the present study that the application of a swab to the larynx did in some cases induce a productive cough.

Anaerobic culture was not employed routinely. In one case numerous pneumococci were present in a film stained by Gram's stain but culture of the specimen under anerobic

conditions produced no growth of pneumococci. When culture was repeated after 24 hours under anaerobic conditions, a profuse growth of type 23 pneumococci was obtained. The pneumococcus obtained by mouse inoculation also required anaerobic conditions of culture. Humphrey and colleagues (1948) considered that anaerobic culture increased the isolation rate of pneumococci.

The finding in the present study emphasises the importance of a preliminary Gram film in the investigation of cases of pneumonia. It also demonstrates that pneumococci can survive in room temperature for 24 hours. Humphrey and others (1948) did not considered pneumococci survived well at this temperature.

It would appear that ideally all specimens should be cultured under anaerobic conditions. This practice however may be limited by the laboratory facilities available and the number of specimens requiring examination. Where conditions preclude anaerobic culture of every specimen, the possibility of an anaerobic organism should be borne in mind when a pneumococcus which is present in a Gram film fails to grow on aerobic culture.

It is apparent from the preceding discussion that in cases of pneumonia the isolation rate of pathogenic bacteria can be

markedly increased by the introduction of comparatively simple bacteriological techniques. It is not considered that any of the methods are beyond the scope of a routine laboratory. The work entailed is not excessive and is far outweighed by the advantage of an early correct diagnosis of the infecting agent.

The use of these techniques does not apply only to the diagnosis of pneumonia, but to all bacteriological examinations of the sputum.

As these methods are mainly concerned with the isolation of pneumococci and Haemophilus influenzae, they could be profitably applied to the examination of the sputum of patients with chronic bronchitis, where these organisms are commonly associated with infection.

It is perhaps fitting at this time to consider the point frequently raised by physicians, that the isolation rate of pathogenic organisms increases when an investigation of a special nature is undertaken. This is the natural result of close co-operation between the laboratory, and clinical and nursing staff, and should not be restricted to such investigations. When all available information and a suitable specimen are sent to the laboratory, the bacteriologist then has the material on which to conduct a full and possible rewarding examination.

Mortality

In a study of the fatal cases in this investigation, the most significant factor which emerged was the high incidence of pneumococcal infections. This organism was definitely present in seven and probably present in another of the eleven fatal cases. It would appear that the mortality rate in pneumococcal pneumonias was about 10%. When other investigations of pneumococcal pneumonia are considered it can be seen that the figure obtained in the present study was relatively high.

Dowling and colleagues (1947) reported a 7% mortality rate. The death rate reported by Austrian and Rosenblum (1953) was 3% and that found by Davis (1954) was 0.5%. 5% of cases of pneumococcal pneumonia studied by Humphrey and colleagues (1948) terminated fatally.

Pneumococci are generally considered to be extremely sensitive to the action of antibiotics and in view of this it is pertinant to enquire further for a possible explanation of the deaths which occurred among patients who received the appropriate therapy.

Type 3 was the predominant pneumococcal type isolated from fatal cases. The high mortality of type 3 pneumococci infections for older people

is demonstrated by the finding that four of the five patients over 60 years infected with this type, had a fatal illness. The isolation of type 3 pneumococci from older patients should be regarded as a finding of serious significance. In the pre-antibiotic era, Heffron (1939) considered that type 3 infections were responsible for 45% to 60% of deaths in pneumococcal pneumonia. This high mortality would appear to still apply to the older age groups who despite antibiotic treatment are unable to withstand infection with this type.

The poor prognosis of pneumonia where there is associated bacteraemia is illustrated by the presence of this condition in three of these four cases of type 3 infections and in one where the infecting strain was a type 14 pneumococcus. This finding would indicate the value of blood culture as a means of assessing the significance of such pneumococcal infections and the possible progress of the disease.

The study demonstrates the extremely grave illness which may result from infection with the pneumococcus; this was evident in one case where the infecting strain was a type 3 pneumococcus with an reduced sensitivity to penicillin. Here the patient suffered an

overwhelming infection, which was manifested by extensive consolidation and the presence of bacteraemia. The other fulminating case was a young girl of 17 years. Although the infecting agent was not proved beyond doubt to be a pneumococcus, it was considered a possible cause in view of the presence of capsulated diplococci in the film of the laryngeal swab, and the clinical appearance of the case.

It was of interest that three weeks previously she had suffered from an infection of the upper respiratory tract, a condition which frequently precedes pneumonia. Straker and colleagues (1939) found an slightly increased carrier rate of pneumococci a week after the onset of a 'cold'. There were therefore in this case possible predisposing conditions for a pneumococcal infection.

It is unfortunate due to failure in cultural technique and omission of culture of the blood, that positive identification of the infecting organism was not made. Although no therapeutic measures were of avail in this case, the identification of an organism which caused an illness of such severity is of the greatest importance.

The possible predisposing action of an upper respiratory infection is demonstrated

in the case infected with a type 6 pneumococcus which is a strain commonly found in the carrier state (Cruickshank and others 1960). Here the patient was an elderly debilitated woman who developed pneumonia two weeks after a cold and this case would seem to have all the features of an endogenous infection.

In the present study there appeared to be a relationship between mortality and increased resistance to penicillin by pneumococci. In two cases this was associated with infection with type 3 pneumococci. The first of these was the case where infection was very severe in type and the patient died 12 hours after admission. Infection in the second case was with a penicillin sensitive pneumococcus which disappeared from the sputum to be replaced by a Gram positive diplococcus which was highly resistant to penicillin, avirulent to a mouse and to the patient. By mouse inoculation this organism was transformed to a type 3 pneumococcus which was highly resistant to penicillin. It is not considered that the penicillin resistant organism isolated at later examinations was itself virulent, but that the type 3 pneumococcus which developed this form of resistance was responsible for a severe infection.

In the third case the penicillin resistant penumococcus was not typed and was isolated from a patient who had a recurrence of a pneumococcal infection. As this case was complicated by the presence of a sarcoma and chronic bronchitis, it is not clear what part was played by the pneumococcal infection in the fatal outcome of the illness. It would appear that it was responsible for the marked inflammatory condition which was present.

An observation of this nature has not been made previously. As the sensitivity of the pneumococcus to penicillin is assumed, treatment of a pneumococcal pneumonia may be commenced without prior determination of the sensitivity of the organism. Austrian (1957) considered that sensitivity testing of pneumococci was not necessary. The findings of the present study refute this. The conclusions from this investigation are that pneumococci are no longer completely sensitive to penicillin and in view of the serious nature of the infection produced by certain resistant strains, it is essential for the sensitivity of the pneumococcus to penicillin to be estimated. If this is found to be reduced it would be advisable that another form of therapy be instituted.

From one case no bacterial pathogens were isolated. Antibiotic treatment had been given before admission which could account for the failure to isolate the infecting organisms. This patient suffered from severe chronic bronchitis. It would appear in this case that the pneumonia was the terminal event in a patient severely disabled by chronic bronchitis.

In this group Staphylococcus aureus was observed as a secondary infecting agent in two patients with chronic bronchitis. The infection in these cases was controlled, but there can be little doubt that the occurrence of pneumonia had a deleterious effect on the progress of the chronic disease. The danger which infection constitutes to the patient with chronic bronchitis is demonstrated by the presence of five such patients in this group of eleven fatal cases.

Summary

153 unselected cases of pneumonia were studied. In 68 cases the pneumococcus alone or with Haemophilus influenzae was the infecting agent. Of the remaining cases 15 were infected by Haemophilus influenzae, 11 by Staphylococcus aureus and in 59 cases no specific bacterial agent was found.

There were 109 male patients and 44 female patients. 69% of the male patients and 59% of the female patients were over 50 years of age.

There was a tendency for pneumococcal infections to occur in the older patients. This was particularly marked in male patients where 78% of these infections were in patients over 50 years.

The pneumococcal types isolated most commonly from older patients were type 3 and those types belonging to the higher numbers. In older patients infection with types 1 to 8 generally produced a more serious illness than when the infecting types were of the higher numbers, also older patients infected with types 1 to 8 had a more severe illness than younger patients infected with these types.

The presence of bacteraemia was a serious prognostic sign in older patients.

An unusual feature was the high incidence of infections considered due to Haemophilus influenzae and the failure to identify a definite predisposing cause in some of these cases.

The commonest predisposing factor to infection with Staphylococcus aureus was the presence of chronic bronchitis and the two deaths in this group occurred in patients with this disease.

31 cases where no specific bacterial agent was isolated had received antibiotic treatment before admission. In a few of the remaining cases it was possible that organisms of low pathogenicity were responsible for infection. In other cases the bacteriological examinations undertaken were incomplete.

The use of selective cultural techniques increased the isolation rate of pathogenic bacteria. 14 strains of pneumococci were isolated by mouse inoculation only.

Strains of pneumococci with an increased resistance to penicillin were isolated. Some were isolated at the start of the pneumonia from patients who had not received penicillin treatment and others were found in the later stages of pneumococcal infections which had been treated with penicillin. Two types of organisms were isolated. One group retained

the morphological and colonial appearance of the pneumococcus but lost the typical biochemical reactions of that organism. Such organisms were found in both the early and late stages of the pneumonia. The second type found in the later stages of pneumonia, were small acapsulated Gram-positive dipococci which did not possess the biochemical reactions of pneumococci.

As it was considered these organisms might be 'rough' strains of pneumococci produced by the action of penicillin *in vivo*, attempts were made to reverse them to capsulated strains by subcutaneous inoculation of mice. Two such experiments were successful in producing a type specific pneumococcus with a reduced sensitivity to penicillin. *In vitro* resistance to penicillin was induced in 30 strains of pneumococci. Pneumococci thus treated lost their colonial and morphological appearance and biochemical reactions, and there was a similarity between the orga isms produced by this form of resistance and those Gram positive diplococci isolated during the later stages of pneumococcal pneumonias. Some strains of pneumococci and *Haemophilus influenzae* showed an increased resistance to tetracycline, a finding which appeared of significance in view of the use of this drug in the treatment of chronic bronchitis.

Pneumococcal infections were responsible for the largest number of deaths in the series and in some fatal cases the strains of pneumococci showed resistance to penicillin. In five of the eleven fatal cases in the series infection was secondary to a previous chronic bronchitic condition.

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